UNIVERSITÉ DU QUÉBEC EN OUTAOUAIS

HISTOIRE ÉVOLUTIVE DE LA SÉRIE SACCHARODENDRON ET CAPACITÉ D'ADAPTATION DE L'ÉRABLE À SUCRE FACE AUX CHANGEMENTS CLIMATIQUES

THÈSE

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DÉDICACE

À mon grand-père, René Pugeaut

AVANT-PROPOS

Les travaux présentés dans cette thèse ont été menés sur des terres faisant partie des territoires ancestraux non cédés de la nation algonquine, Omàmiwininìwag.

Cette recherche a été dirigée par le Professeur Yann Surget-Groba à l'Université du Québec en Outaouais et a bénéficié d'un financement du Conseil de Recherche en Sciences Naturelles et en Génie (CRSNG) du Canada.

Cette thèse est constituée d'une introduction générale, de trois chapitres constituant le corps de la thèse (chapitres 1, 2, 3) et d'une conclusion générale. Les trois chapitres du corps de la thèse correspondent chacun à un manuscrit destiné à une publication scientifique.

Le premier chapitre explore les relations phylogénétiques au sein du genre *Acer*, en s'intéressant plus particulièrement aux lignées évolutives de la série *Saccharodendron*, qui inclut l'érable à sucre. Cette étude évalue également l'efficacité de l'approche de séquençage à faible profondeur (‘*genome skimming*’) couplée à une méthode de reconstruction phylogénétique rapide ne nécessitant ni assemblage ni alignement des séquences. Le manuscrit sera soumis à une revue scientifique après l'ajout d'une section approfondissant l'évaluation de cette méthodologie, afin d'en renforcer la portée et la robustesse.

Le deuxième chapitre porte sur la délimitation génétique entre l'érable à sucre et son plus proche parent sympatrique, l'érable noir. Le manuscrit correspondant a été soumis à l'*American Journal of Botany* et est en cours d'évaluation par les pairs.

Le troisième chapitre examine les patrons d'adaptation locale au sein de populations d'érable à sucre au Québec. Le manuscrit, tel qu'il est présenté dans cette thèse, est en cours de relecture par les collaborateurs du projet, Nathalie Isabel et Martin Perron. Une fois révisé, il pourra être soumis pour publication.

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LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

AAF : Assembly-Alignment-Free

BC : Bias Corrected

bc : base coverage

BSME : type de couvert végétal « Black spruce with moss or ericaceous »

c : coverage

CEC.clay : capacité d'échange cationique de la portion argileuse d'un sol

cpDNA : chloroplastic DNA

CRSNG : Conseil de Recherche en Sciences Naturelles et en Génie

CTAB : bromure de cétyltriméthylammonium

DNA : desoxyribonucleic acid

DAPC : Discriminant Analysis of Principal Components

e.g. : *exempli gratia*

EDTA : Ethylenediaminetetraacetic acid

ESU : Evolutionarily Significant Units

F : coefficient de consanguinité

FAO : Food & Agriculture Organization

FDR : False Discovery Rate

F_{IS} : Indice de fixation

F_{ST} : Indice de différenciation

GEA : Genome-Environment Association

GBS : Genotype-By-Sequencing

GC content : Proportion d'un brin d'ADN en guanine et cytosine.

GDSL : Glycine-Aspartate-Sérine-Leucine

gs : genome size

He : hétérozygotie attendue sous l'équilibre d'Hardy-Weinberg

Ho : hétérozygotie observée

IBD : Isolation By Distance

IBIS : Institut de Biologie Intégrative et des Systèmes

i.e. : *id est*

IIASA : International Institute for Applied Systems Analysis

IUCN : International Union for Conservation of Nature

K : nombre de groupes génétiques ou de populations ancestrales

k-mer : Fragment de longueur k dans une séquence d'ADN

LGM : Last Glacial Maximum

Ma : Millions d'années

MAF : Minor Allele Frequency

Max : maximum

MCMC : Markov chain Monte Carlo

Min : minimum

ML : type de couvert végétal « Maple bush with linden »

MRNF : Ministère des Ressources Naturelles et des Forêts

MYBB : type de couvert végétal « Maple bush with yellow birch or beech »

NGS : Next Generation Sequencing

OA : outliers' approach

PCA : Principal Component Analysis

PCR : Polymerase Chain Reaction

ph : distribution théorique des k-mers

pH : potentiel hydrogène

p-value : significativité statistique

q-value : p-value ajustées pour les faux positifs

RAPD : Random Amplified Polymorphic DNA

RDA : Redundancy Analysis

SNP : Single Nucleotide Polymorphism

s.e. : standard error

TE : Tris-EDTA

USDA : United States Department of Agriculture

VIF : Variance Inflation Factor

YBF : type de couvert végétal « Yellow birch grove with fir or fir grove with yellow birch »

z-score : mesure statistique de la relation entre la valeur et la moyenne d'un groupe de valeurs

LISTE DES SYMBOLES ET DES UNITÉS

% : Pourcentage

° : Degré

°C : Degré Celsius

$_{adj}R^2$: coefficient de corrélation ajusté

bp : base pair

cmolc : centimol de carga

kg : kilogramme

kJ : kilojoule

km : kilomètre

kPa : kilopascal

cm : centimètre

m : mètre

mg : milligramme

min : minute

mm : millimètre

ng : nanogramme

π : diversité nucléotidique

s : seconde

X : profondeur d'alignement

RÉSUMÉ

Les changements climatiques actuels constituent une menace majeure pour la biodiversité mondiale et les services écosystémiques qui en dépendent. Face à des variations climatiques abruptes, les organismes vivants doivent soit s'adapter aux nouvelles conditions environnementales, soit migrer vers des habitats plus favorables. Cette thèse s'intéresse aux adaptations génomiques liées aux paramètres climatiques chez l'érable à sucre (*Acer saccharum*), une espèce largement répandue dans l'est de l'Amérique du Nord, d'une importance écologique pour les forêts feuillues et mixtes, ainsi que d'un intérêt économique significatif pour les secteurs forestier et agroalimentaire. L'étude de la variation génomique adaptative repose sur différentes méthodes, parmi lesquelles des approches de génomique du paysage. Toutefois, l'hybridation avec des espèces proches et certains événements de l'histoire démographique peuvent produire des signaux génomiques similaires à ceux de l'adaptation, compliquant ainsi la détection de variations génomiques adaptatives par les approches génomiques. Une compréhension précise des niveaux d'hybridation entre l'espèce cible et ses espèces apparentées est donc essentielle. Le premier chapitre de cette thèse explore les relations phylogénétiques entre l'érable à sucre et ses plus proches parents du genre *Acer*, en particulier les érables de la série *Saccharodendron* (*i.e.* plusieurs taxons d'érables distribués dans l'est de l'Amérique du Nord). Le deuxième chapitre approfondit la distinction génétique entre l'érable à sucre et son plus proche parent, l'érable noir (*Acer nigrum*), notamment en raison de leur sympatrie au sud du Québec et de leur réputation d'hybridation extensive. L'étude de cette délinéation génétique était nécessaire, car certaines populations analysées dans le cadre de la détection des variations génomiques adaptatives se situent en zone de sympatrie avec l'érable noir. Enfin, le troisième chapitre présente l'utilisation d'approches de génomique du paysage pour identifier les variations génomiques impliquées dans l'adaptation locale des populations d'érable à sucre au Québec. Les résultats de cette thèse mettent en évidence l'existence de lignées évolutives distinctes au sein des membres de la série *Saccharodendron*. De plus, les analyses génétiques démontrent une distinction claire entre l'érable à sucre et l'érable noir, même à une échelle géographique très fine (~1 km). Ces résultats permettent d'écarter l'hypothèse selon laquelle l'hybridation interspécifique exercerait une

influence significative sur la distribution de la variation génomique des populations d'érable à sucre au Québec. Le troisième chapitre discute des traces laissées par les oscillations climatiques du Quaternaire sur la distribution actuelle de la variation génomique de l'érable à sucre et atteint l'objectif principal de cette thèse en identifiant les variations génomiques associées à l'adaptation locale. Bien que certaines limites soient discutées dans chacun des chapitres et en conclusion générale, ces travaux contribuent à confirmer ou à réfuter certaines hypothèses préexistantes concernant cette espèce emblématique d'Amérique du Nord. D'une part, cette thèse infirme l'hypothèse selon laquelle les érables de la série *Saccharodendron* constitueraient un ensemble de sous-espèces, une question encore débattue dans la communauté scientifique en raison du manque d'études explicitement consacrées à cette problématique. D'autre part, les résultats du dernier chapitre corroborent certaines hypothèses antérieures sur l'érable à sucre : (i) une faible différenciation génétique entre populations, indiquant un flux génique élevé et confirmant la pollinisation anémochore de l'espèce, (ii) une diversité génétique élevée et homogènement distribuée dans le paysage. Enfin, cette thèse identifie des variations génomiques associées à l'adaptation à l'environnement abiotique, apportant des éléments essentiels à la compréhension de la capacité adaptative de l'érable à sucre. En conclusion, les résultats de cette thèse enrichissent nos connaissances sur l'érable à sucre et ses espèces apparentées, fournissant ainsi des données utiles à l'amélioration des stratégies de conservation et de gestion d'espèces emblématiques des forêts tempérées.

Mots clés : génomique du paysage, phylogénie, érable à sucre, capacité d'adaptation, délinéation d'espèces, génomique de la conservation.

ABSTRACT

Current climate change poses a threat to global biodiversity and the ecosystem services associated with it. When climatic conditions change abruptly, living organisms can either adapt to their new environment or migrate to a more favorable habitat. This thesis investigates the genomic adaptations related to climatic parameters in the sugar maple (*Acer saccharum*), a species widely distributed in eastern North America that plays a crucial ecological role in deciduous and mixed forests and holds significant economic value in the forestry and agri-food sectors. The detection of adaptive genomic variations can be achieved through a landscape genomics approach. However, hybridization with closely related species and certain events in a species' demographic history can produce genomic signals like those resulting from adaptation. Thus, detecting adaptive genomic variations requires a better understanding of hybridization levels between the target species and its close relatives. The first chapter of this thesis explores the phylogenetic relationships of the sugar maple with its closest relatives within the genus *Acer*, specifically the maples of the *Saccharodendron* series (*i.e.* several maple taxa distributed in eastern North America). In the second chapter, the genetic delineation between the sugar maple and its closest relative, the black maple (*Acer nigrum*), is examined in greater detail. This in-depth investigation was justified by the fact that these two species coexist in sympatry, particularly in southern Quebec. Moreover, these maples are reputed to hybridize extensively. Since some sugar maple populations studied for detecting adaptive genomic variation were in sympatric zones with black maple, it was necessary to further explore their genetic delineation. Finally, the third chapter describes the use of landscape genomics approaches to study the genomic variation involved in the local adaptation of sugar maple populations in Quebec. The results of this thesis first highlight distinct evolutionary lineages among members of the *Saccharodendron* series. Furthermore, the second chapter demonstrates genetic delineation between the sugar maple and the black maple, even at a very small geographical scale (~1 km). The combined results of these first two chapters allowed us to rule out the hypothesis of a potential influence of interspecific hybridization on the distribution of genomic variation within sugar maple populations in Quebec. The third chapter, in turn, discusses the imprints left by Quaternary climatic oscillations on the

current distribution of genomic variation in sugar maple. Ultimately, this final chapter achieves the thesis's objective of highlighting genomic variation involved in local adaptation in sugar maple. Despite certain limitations outlined in each chapter and the general conclusion, the results of this thesis contribute to confirming or refuting established facts about sugar maple, an iconic North American species. First, they refute a hypothesis present in the scientific literature suggesting that the maples of the *Saccharodendron* series constitute a set of subspecies. While this hypothesis is not widely accepted within the scientific community, very few studies provide concrete results to specifically address it. Indeed, most studies merely adopt a classification approach (*i.e.*, species, subspecies, or varieties) and focus on other research questions concerning maples. Additionally, the results of the final chapter confirm hypotheses previously proposed regarding sugar maple. First, we observed low levels of genetic differentiation between populations, suggesting high levels of gene flow and providing evidence for the wind pollination mechanism of this species. Second, sugar maple exhibits high genetic diversity, evenly distributed across the landscape. Finally, the detection of genomic variation associated with adaptation to abiotic environment in this species contributes to characterizing its adaptive capacity. Overall, the findings of this thesis enhance our understanding of sugar maple and its related species, providing valuable knowledge for the effectiveness of conservation plans and strategies for maintaining temperate forests.

Keywords : landscape genomics, phylogeny, sugar maple, adaptability, species delineation, conservation genomics.

Introduction générale

0.1 Contexte général

0.1.1 L'écosystème des forêts tempérées

Les forêts tempérées, réparties sur cinq grandes régions du globe, sont des écosystèmes d'une importance cruciale en raison de la multitude de services écologiques, économiques et sociaux qu'ils fournissent (de Gouvenain & Silander, 2017; Glenn-Lewin, 1977; Huntley, 1993). Tout d'abord, ces écosystèmes constituent des réservoirs de biodiversité structurelle, fonctionnelle et phylogénétique du fait de la variété d'espèces animales et végétales qu'ils abritent (Wilson & Peter, 1988). Les différentes composantes de la biodiversité des forêts tempérées interagissent dans des réseaux écologiques complexes qui fournissent de nombreux services écosystémiques comme la pollinisation, la dispersion de graines et la production de biomasse (Brockerhoff et al., 2017). De plus, comme les autres écosystèmes forestiers, ils ont des rôles fondamentaux dans les cycles de certains éléments essentiels (Currie & Bergen, 2008; Lindquist & D'Annunzio, 2016). En premier lieu, les forêts tempérées participent au stockage de grandes quantités de carbone atmosphérique sous forme de biomasse vivante et de matière organique morte (Gower, 2003). Ce processus contribue ainsi à atténuer les effets des changements climatiques induits par les activités anthropiques (Lal & Lorenz, 2012). De plus, elles influencent le cycle de l'eau en régulant les précipitations et en réduisant l'érosion des sols, ce qui est particulièrement pertinent dans les zones sujettes à des pressions anthropiques croissantes (Brockerhoff et al., 2017). Enfin, elles jouent également un rôle essentiel dans le cycle de l'azote en facilitant la fixation, la minéralisation et la décomposition de cet élément (Rennenberg & Dannenmann, 2015). Par ailleurs, les forêts tempérées offrent des ressources essentielles pour les sociétés humaines. Elles fournissent en effet des ressources alimentaires et des espaces récréatifs qui participent à l'amélioration de la qualité de vie (Bang et al., 2017; Dlamini, 2020). Elles représentent également une source majeure de bois et de produits non ligneux exploités par les sociétés humaines à l'échelle mondiale (Fusco et al., 2024). Bien que les forêts tempérées représentent environ 16 % des forêts mondiales, elles ont la plus faible densité forestière par habitant, car elles se trouvent généralement dans des régions fortement peuplées (Gilliam, 2016; Hansen et al., 2010). En

conséquence, seulement environ 1 % des forêts feuillues tempérées actuelles de l'hémisphère Nord sont considérées comme des forêts anciennes intactes (Silander, 2001; Strobel, 1997), tandis que la majorité sont soit exploitées pour la production de bois, soit converties en plantations, soit modifiées par les pratiques humaines.

0.1.2 Changements globaux et forêts tempérées

En plus de l'exploitation intensive, le fonctionnement et la pérennité des forêts tempérées sont mis en péril par des perturbations biotiques et abiotiques à l'échelle mondiale causées par les changements globaux (Fusco et al., 2024; Trumbore et al., 2015). En Amérique du Nord, considérée comme l'un des trois principaux foyers de l'écosystème des forêts tempérées (Fischer et al., 2013), les projections climatiques prévoient une augmentation des températures moyennes annuelles ainsi que des changements dans les régimes de précipitations au cours des prochaines décennies (Liu et al., 2017). Ces changements de conditions environnementales impactent directement les espèces des forêts tempérées, en particulier les arbres, qui constituent la base de ces écosystèmes (Aitken, Yeaman, Holliday, Wang, & Curtis-McLane, 2008; Aubin et al., 2018). En effet, de nombreuses espèces d'arbres migrent vers des latitudes ou altitudes plus élevées à mesure que les températures augmentent (Boisvert-Marsh et al., 2014; McKenney et al., 2007; Thuiller, 2004). Ces changements d'aire de répartition modifient l'abondance des espèces et la composition des communautés, et par conséquent les processus et services écosystémiques de la forêt tempérée (Putten, 2012). Par ailleurs il est attendu que certaines populations (ou espèces) ne parviennent pas à effectuer ces changements de distribution par la migration et que leur maintien dépende uniquement de leur capacité à s'adapter aux nouvelles conditions, ce qui augmente le risque d'extinction locale pour les espèces forestières (Aubin et al., 2016). Par ailleurs, une augmentation de la fréquence et de l'intensité des événements climatiques extrêmes, tels que sécheresses, incendies, tempêtes de verglas, cyclones et inondations, est également anticipée (Pachauri et al. 2014). Ces épisodes climatiques extrêmes, notamment les sécheresses, diminuent la vigueur des populations et augmentent également les taux de mortalité des arbres (Allen et al., 2015; Andivia et al., 2020). Parallèlement, la pollution

de l'air et l'introduction d'espèces invasives aggravent les pressions exercées sur ces écosystèmes (Blackburn et al., 2011; Gundersen et al., 2006). En effet, les espèces invasives peuvent avoir des effets dévastateurs sur les espèces, comme par exemple le cas de l'agrile du frêne qui a décimé des millions d'arbres en Amérique du Nord (Herms & McCullough, 2014). Ces phénomènes individuels et leur combinaison sont ainsi associés avec le déclin de la vigueur et de la taille des populations d'espèces forestières en Amérique du Nord. Dans les cas les plus extrêmes ils provoquent des extinctions locales qui mettent gravement en péril l'écosystème de la forêt tempérée (Keenan, 2015).

0.1.3 Comment participer au maintien des forêts tempérées ?

Compte tenu des défis complexes auxquels font face les forêts tempérées dans le contexte des changements globaux, la communauté scientifique explore divers axes de recherche pour évaluer leurs impacts et comprendre les stratégies d'adaptation des espèces forestières. Un premier volet de recherche repose sur la surveillance écologique basée sur des suivis à long terme. Ces études combinent des observations de terrain, des données de télédétection et des modèles climatiques pour documenter l'évolution des conditions environnementales et de l'état des forêts (Anderson-Teixeira et al., 2015; Bussotti & Pollastrini, 2017). Cette approche permet d'identifier les tendances à grande échelle, telles que les changements dans la répartition des espèces, les taux de mortalité des arbres et l'intensité des perturbations naturelles comme les incendies et les tempêtes. Un second volet de recherche vise à explorer les réponses biologiques aux changements environnementaux en étudiant les différents niveaux de biodiversité : des individus aux populations, des communautés d'espèces aux écosystèmes. Cette approche permet de mieux comprendre comment les espèces s'adaptent aux variations climatiques passées et présentes, tout en modélisant leurs réponses futures. Les stratégies d'adaptation étudiées incluent les déplacements géographiques, les ajustements phénologiques (comme la floraison et la production de graines), les mécanismes moléculaires et physiologiques favorisant la tolérance aux stress climatiques et la capacité d'adaptation des espèces (Locatelli et al., 2010). Ces connaissances soutiennent l'élaboration de plans de gestion durable, en guidant les décisions sur

la conservation, la restauration et l'exploitation responsable des forêts tempérées (Fischer et al., 2013; Park et al., 2014). La thèse proposée dans ce document s'inscrit dans ce second axe de recherche en se concentrant sur l'évaluation de la capacité d'adaptation aux variations climatiques d'une espèce emblématique des forêts tempérées d'Amérique du Nord : l'érable à sucre (*Acer saccharum*, Marshall). En raison de son importance écologique, économique et culturelle, cette espèce peut servir de modèle pour la compréhension des conséquences écologiques et sociales des changements climatiques sur les forêts tempérées. L'analyse des réponses adaptatives de l'érable à sucre offre des perspectives essentielles pour anticiper les transformations des paysages forestiers et proposer des stratégies de gestion adaptées aux défis environnementaux actuels.

0.2 L'adaptation des arbres au climat

0.2.1 La capacité d'adaptation

L'évolution des organismes se matérialise par des changements de fréquences alléliques entre les générations. Il existe quatre forces évolutives influençant les fréquences alléliques : i) la mutation génétique par laquelle de nouveaux allèles sont créés aléatoirement, ii) la dérive génétique qui fixe des allèles dans les populations, iii) le flux génique qui introduit des allèles venant de populations extérieures par la migration et iv) la sélection naturelle par laquelle les allèles conférant un avantage sélectif vont être plus représentés dans les générations suivantes. La sélection naturelle est la force à l'origine de l'adaptation des organismes à leur environnement, elle agit cependant de manière conjointe avec les autres forces évolutives pour façonner en continu la forme, le nombre et la position géographique des gènes, individus, populations et espèces. Dans le contexte actuel des changements globaux, le maintien des organismes dans leur environnement est mis en péril, car les changements de conditions environnementales, biotiques et abiotiques, bouleversent l'équilibre naturel des forces évolutives (Habibullah et al., 2022; Rinawati et al., 2013). Pour survivre dans un nouveau contexte environnemental, les organismes dépendent de leur capacité d'adaptation qui est définie par Nicotra et al. (2015) comme « la

capacité d'une espèce ou de ses populations à faire face à un changement donné ou à y répondre en persistant *in situ* ou en se déplaçant vers des aires de répartition ou des microhabitats plus appropriés » (Beever et al., 2016; Dawson et al., 2011). Selon cette définition, la capacité d'adaptation peut être déconstruite en trois composantes principales (qui ne s'excluent pas nécessairement mutuellement) : (i) des caractéristiques démographiques ou de l'histoire de vie, y compris les capacités de dispersion et de colonisation; (ii) la diversité génétique et le potentiel adaptatif par sélection naturelle; et (iii) la plasticité phénotypique, y compris l'acclimatation physiologique. Cette définition englobe donc tous les processus génétiques, épigénétiques et d'acclimatation qui participent au maintien des organismes dans de nouvelles conditions environnementales et qui sont mesurables à différentes échelles (*e.g.* la plasticité phénotypique au niveau individuel et le potentiel adaptatif au niveau populationnel) (Figure 0.1). Ainsi, selon Nicotra et al. (2015), cette thèse participe à l'évaluation d'une composante de la capacité d'adaptation de l'érable à sucre en se concentrant sur sa diversité génétique et son potentiel adaptatif.

0.2.2 Le cas particulier des arbres

Les arbres sont des espèces au cycle de vie long qui migrent lentement, car ce ne sont pas les individus eux-mêmes, mais leur descendance (*i.e.* les graines) qui se déplacent. Dans ce contexte particulier, il est attendu que les arbres aient des taux migratoires plus lents que la rapidité des changements climatiques, ce qui peut impliquer que certaines populations seront mésadaptées à leur environnement dans les prochaines années (Bisbing et al., 2021; Capblancq, Fitzpatrick, et al., 2020; de Lafontaine et al., 2018). Ainsi, dans le but de mieux comprendre les ressources adaptatives des arbres, la documentation de la diversité génétique liée à l'adaptation aux variations environnementales chez les espèces d'arbres a connu un essor considérable dans les dernières années (Alakärppä et al., 2018; Isabel et al., 2020).

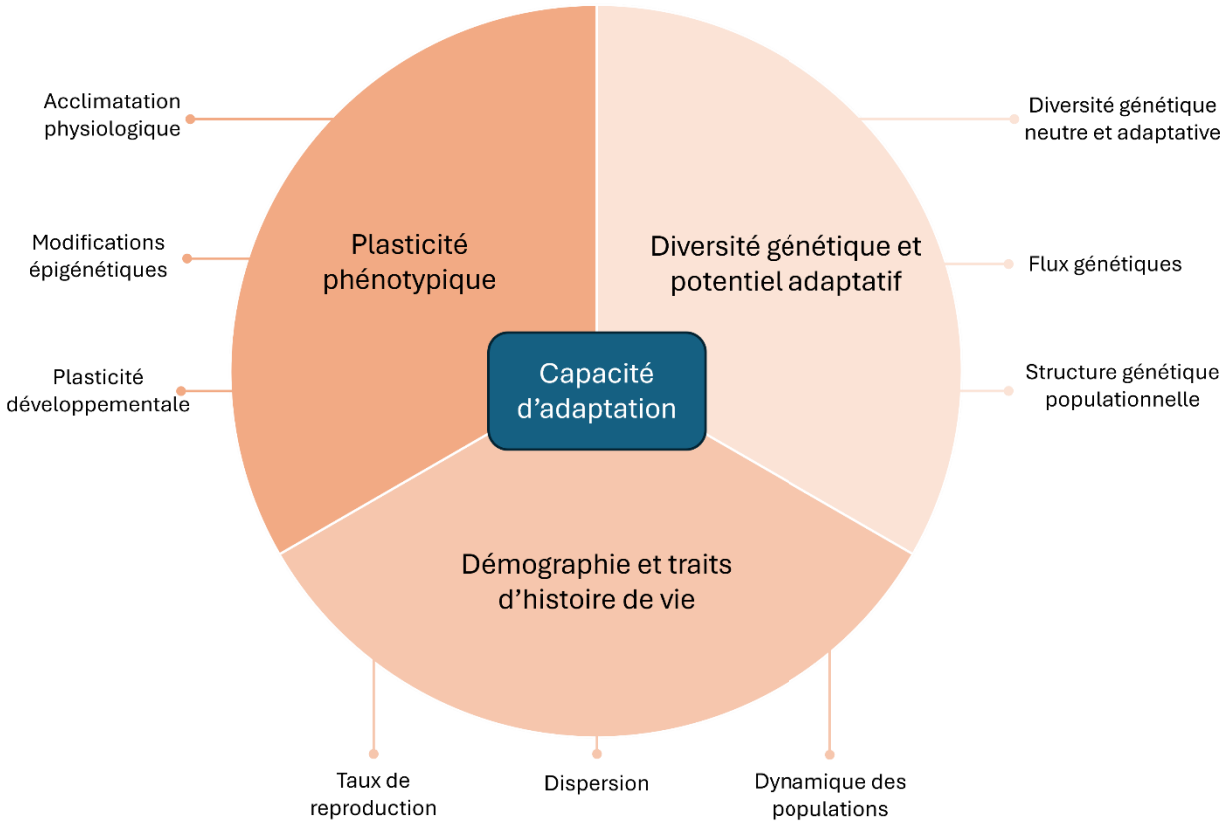

Figure 0.1.1 : Schéma conceptuel inspiré des composantes de la capacité d'adaptation décrites par Nicotra et al. (2015).

Actuellement, il est consensuel que le maintien des populations d'arbres dans un nouveau contexte environnemental dépend en grande partie de la diversité génétique préexistante ('*standing genetic variation*') qui sert de base pour l'acclimatation et l'adaptation rapide (*i.e.* sur quelques générations) (Ahrens et al., 2019; Barrett & Schluter, 2008; Capblancq, Morin, et al., 2020; Hamrick, 2004). Afin d'aller plus loin, de nombreuses méthodes ont été développées pour identifier les parts adaptative (*i.e.* impliquée dans l'adaptation) et neutre (*i.e.* non impliquée dans l'adaptation) de la variation génétique préexistante dans les populations naturelles (Ahrens et al., 2018; Joost et al., 2007; Lotterhos & Whitlock, 2015). Au-delà de la quantification de la diversité génétique préexistante, l'identification plus précise de la variation génétique liée à l'adaptation a des applications pratiques, car elle offre une information précieuse pour la mise en place de plans

de conservation des espèces forestières, comme la migration assistée de génotypes adaptés aux conditions futures (Aitken & Bemmels, 2016; Aitken & Whitlock, 2013).

0.2.3 L'adaptation locale

0.2.3.1 Les jardins communs

Dans le but de préserver les espèces d'arbres, piliers des forêts tempérées, la communauté scientifique cherche à comprendre les bases génétiques de leur adaptation au climat. Selon Kawecki et Ebert (2004), l'adaptation locale se définit comme une meilleure valeur sélective des individus locaux par rapport aux individus provenant d'un autre milieu. Ainsi, un bon moyen de détecter l'adaptation locale est de transférer des individus de diverses sources environnementales (*i.e.* provenances) dans un nouvel environnement (*i.e.* jardin commun) et d'évaluer si les individus locaux sont plus aptes que les étrangers (Blanquart et al., 2013a; Schwinning et al., 2022). Dans le cas des arbres, il est souvent impossible de mesurer la valeur sélective des individus telle que définie par Hunt et Hodgson (2010), *i.e.* la proportion de descendants qui atteignent la maturité sexuelle dans la génération suivante, dans les délais d'une étude scientifique standard. Par conséquent, les jardins communs pour les arbres mesurent généralement des traits phénotypiques ou physiologiques, ayant un lien direct ou indirect avec la valeur sélective, pour évaluer la performance des individus dans l'environnement commun. Les traits adaptatifs identifiés peuvent être ensuite associés à des positions génétiques (Capblancq, 2023; Cavender-Bares & Ramírez-Valiente, 2017; de Villemereuil et al., 2016; Eckert et al., 2015). Néanmoins, étant donné l'architecture génétique complexe de certains traits phénotypiques, l'identification de la variation génétique adaptative peut se révéler fastidieuse, en particulier dans le cas des espèces non-modèles pour lesquelles aucun génome de référence n'est disponible. Pour pallier ces complications, des méthodes de génomique du paysage peuvent être appliquées afin d'identifier de la variation génomique associée à des gradients environnementaux sans nécessairement effectuer une expérience de jardin commun (Hendricks et al., 2018).

0.2.3.2 Génomique du paysage

Dans les dernières années, l'essor des technologies de séquençage à haut débit a permis à la communauté scientifique d'accéder à une très grande quantité de données génétiques pour un coût relativement modeste. Grâce à ce type de données, il est possible de capturer une image actuelle de la variation génomique (*i.e.* variation génétique à l'échelle du génome entier) présente aux seins des populations naturelles. Enfin, des méthodes de génomique du paysage ont été développées pour associer statistiquement des patrons de distribution de la variation génomique avec des gradients environnementaux (Bragg et al., 2015; Rellstab et al., 2015). Ces méthodes ont été très utilisées pour détecter l'adaptation locale chez les arbres et ont mis en évidence la variation génomique associée à l'adaptation au climat chez plusieurs espèces forestières (Capblancq, Morin, et al., 2020; Gram & Sork, 2001; Pluess et al., 2016). Ces méthodes sont basées sur la théorie des forces évolutives ; du fait de l'action de la sélection naturelle, les fréquences alléliques varient le long des gradients environnementaux (Sork & Smouse, 2006). Cependant, comme explicité précédemment, les changements dans les fréquences alléliques peuvent être influencés par d'autres processus évolutifs comme les flux de gènes et la dérive génétique. De ce fait les patrons de distribution des fréquences alléliques dans le paysage peuvent être façonnés par d'autres processus que l'adaptation. Dans le cas de l'érable à sucre il convient d'étudier deux phénomènes qui pourraient affecter la distribution des fréquences alléliques.

En premier lieu, il est nécessaire d'explorer l'hybridation avec des espèces proches. En effet, l'hybridation entre espèces proches introduit de nouveaux allèles dans les populations et si les hybrides sont viables cela peut provoquer des changements de fréquences alléliques dans les générations suivantes. Dans le cas de l'érable à sucre, il est donc essentiel de considérer d'autres espèces d'érables distribuées dans l'est Amérique du Nord. En particulier, l'érable à sucre est très proche phylogénétiquement des érables faisant partie de la même série taxonomique que lui (appelée série *Saccharodendron*) et il est supposé s'hybrider facilement avec eux (Dansereau & Desmarais, 1947; Jackson et al., 2021).

En deuxième lieu, il est important de souligner que plusieurs espèces d'arbres de l'est d'Amérique du Nord présentent une structure génétique (*i.e.* une distribution non-homogène de la variation

génomique à travers le paysage) qui serait imputable à l'histoire géologique de la région (Bemmels & Dick, 2018; Jaramillo-Correa et al., 2009; Roberts & Hamann, 2015). En effet, durant la période du Pléistocène (-2.50 Ma ; -10 000 ans), cette région du monde a été marquée par des évènements de glaciation et de déglaciation caractérisés par des avancées et retraits successifs de glaciers. Ces évènements ont impliqué des mouvements de migrations chez les espèces présentes à l'époque et des évènements démographiques associés ; par exemple l'isolement géographique de populations, le contact secondaire de populations préalablement isolées, des goulots d'étranglement, des colonisations rapides. Ces évènements démographiques passés ont laissé des traces dans les patrons de distribution des fréquences alléliques qui peuvent être confondus avec les marques d'adaptation locale (Hewitt, 2004).

Pour ces raisons, la mise en place efficace des méthodes de génomique du paysage pour la caractérisation de la variation génétique en lien avec l'adaptation au climat chez l'érable à sucre nécessite des travaux préalables sur l'histoire évolutive de la série taxonomique *Saccharodendron* qui comprend cette espèce et ses proches parents (Rellstab et al., 2015).

0.3 La série taxonomique *Saccharodendron*

0.3.1 Taxinomie

Sur le plan phylogénétique, l'érable à sucre est étroitement apparenté à cinq autres lignées d'érables d'Amérique du Nord et d'Amérique centrale *(A. floridanum, A. grandidentatum, A. leucoderme, A. nigrum, A. skutchii*). Ces taxons ont été décrits sur des bases morphologiques et sont distribués sur une large aire géographique, du Guatemala jusqu'au Québec et de l'océan Atlantique jusqu'à l'ouest de l'état de l'Arizona aux États-Unis. Ces érables sont regroupés dans la série *Saccharodendron*, au sein de la section *Acer* du genre *Acer* L. de la famille des *Sapindaceae* (Areces-Berazain et al., 2021; Van Gerelden et al., 1994). Dans la littérature, il n'existe pas de consensus sur la désignation taxonomique de ces lignées d'érables, qui sont considérées soit comme des espèces, soit comme des sous-espèces (Harris, Frawley, et al., 2017; Jackson et al., 2021; Van Gerelden et al., 1994). Dans le passé, ces lignées d'érables ont également été

considérées comme des variétés, des écotypes ou des races. Notamment, Howard Kriebel (1957) avait avancé que ces lignées d'érables sont des races d'une seule et même espèce à la morphologie très variable. Au contraire, une étude récente utilisant des microsatellites pour clarifier la structure génétique au sein de cette série taxonomique a suggéré que ces lignées correspondent à des groupes génétiques distincts (Jackson et al., 2021). Cependant, des recherches supplémentaires utilisant des données de séquençage à haut débit sont nécessaires pour clarifier les statuts taxonomiques de ces lignées. Dans le cas de cette thèse, il est particulièrement pertinent d'investiguer les relations entre l'érable à sucre et quatre des érables de la série (*A. floridanum, A. grandidentatum, A. nigrum, A. leucoderme*), car il y a eu observation de phénotypes intermédiaires, *i.e.* des individus présentant un mélange de caractéristiques morphologiques typiques à deux de ces taxons, qui suggèrent de l'hybridation (Dansereau & Desmarais, 1947).

0.3.2 Histoire évolutive

Les archives fossiles et les études phylogénétiques ont permis de dater l'origine du genre *Acer* à environ -60 Ma, durant la période Paléogène au début du Cénozoïque (Areces-Berazain et al., 2021; Gao et al., 2020; Wolfe & Tanai, 1987). Les dernières études biogéographiques sur ce genre s'accordent sur une origine asiatique des érables suivis de mouvements de colonisation vers les continents européens et américains (Areces-Berazain et al., 2021; Gao et al., 2020). Les lignées de la série *Saccharodendron*, majoritairement distribuées sur la côte Est de l'Amérique du Nord, sont plus proches phylogénétiquement d'un groupe d'érables d'Europe que des érables de la côte Ouest de l'Amérique du Nord. Ce patron biogéographique suppose deux évènements de colonisation distincts pour les lignées d'érables de l'Amérique du Nord ; un mouvement de colonisation par le détroit de Béring donnant lieu aux espèces d'érables actuellement distribuées sur la côte Ouest et un mouvement de colonisation à travers l'Europe et l'océan Atlantique donnant lieu aux lignées de la série *Saccharodendron* distribuées sur la côte Est. Cette hypothèse sur la colonisation transatlantique de l'ancêtre de l'érable à sucre est soutenue par l'absence de fossiles de cette série taxonomique dans les régions de l'Alaska et des hautes latitudes de l'Asie

orientale (Wolfe & Tanai, 1987) et leur présence en Islande (Grímsson & Denk, 2007). Un tel évènement de colonisation aurait été rendu possible par la présence du pont terrestre de l'Atlantique Nord de l'Éocène (Tiffney, 1985). D'après les datations phylogénétiques, les taxons de la série *Saccharodendron* aurait commencé à diverger durant le Miocène (environ -15 Ma) (Areces-Berazain et al., 2021). La première divergence correspond à *A. grandidentatum* qui possède la distribution la plus à l'ouest. La divergence de ce taxon est supposée avoir été initiée par l'isolement géographique. Cela fait du sens au regard de plusieurs fossiles attribués à cette série taxonomique qui proviennent de plusieurs localités du début du Miocène dans l'ouest de l'Amérique du Nord (Colombie-Britannique, Oregon, Nevada) (Wolfe & Tanai, 1987) ce qui suggère une répartition plus large en Amérique du Nord à cette époque qu'aujourd'hui. La divergence de *A. skutchii*, datant du Pliocène, serait liée à l'isolement géographique de populations de l'ancêtre des taxons de la série *Saccharodendron* au Mexique et au Guatemala lors des oscillations glaciaires (Vargas-Rodriguez & Platt, 2012). Enfin, les taxons *A. nigrum*, *A. saccharum*, *A. leucoderme* et *A. floridanum* sont supposés avoir divergé au début du Quaternaire mais les causes potentielles de cette divergence (*e.g.* isolation de lignées durant les oscillations glaciaires et adaptation locale à des environnements contrastés) restent, à ce jour, hypothétiques.

La proximité génétique et morphologique de ces taxons ainsi que leur histoire évolutive récente participent à la confusion taxonomique qui règne autour de leur désignation. En effet, la spéciation étant un processus continu, il est actuellement difficile d'estimer si les niveaux de divergences entre ces taxons sont suffisants pour déclarer que la spéciation est définitive.

0.3.3 Continuum de spéciation et concepts d'espèces

Le continuum de spéciation est un concept qui s'applique bien à la série *Saccharodendron*. Ce concept a récemment connu un élan de popularité, mais il puise son origine dans les bases de la biologie évolutive. En effet, dans l'Origine des Espèces et ses autres écrits, Darwin a clairement fait comprendre qu'il pensait à la spéciation comme un processus progressif, se produisant par l'action cumulative de la sélection naturelle. Le concept de continuum trouve donc ses racines

dans cette vision gradualiste de l'évolution et du problème qui en découle. Plus précisément, comme la spéciation se produit généralement sur une échelle de temps bien supérieure à celle d'une durée de vie humaine, nous pouvons rarement observer directement l'ensemble du processus. Les balbutiements du concept de continuum de spéciation sont visibles dans les travaux de Walsh (1864) et Wallace (1865) qui montrent la possibilité de reconstituer des étapes de divergences entre des taxons en comparant des écotypes. Malgré ces premiers exemples de pensée du continuum, le premier usage explicite du terme "continuum de spéciation" a été fait par Drès et Mallet (2002). Les auteurs ont supposé que la transition entre les stades 'polymorphismes', 'races' et 'espèces isolées' de leur modèle biologique était due à une réduction des niveaux d'hybridation. Le concept de continuum est devenu plus populaire avec l'intérêt croissant pour la spéciation écologique et l'interaction entre la divergence adaptative et le flux génétique à la fin des années 2000 (Feder et al., 2012; Nosil, 2008). Dans ce contexte, la synthèse proposée par De Queiroz (2007) a unifié les différents concepts d'espèce existant jusqu'à lors sous une seule définition de l'espèce : celle de lignée évolutive distincte. Selon cette approche, les lignées se distribuent le long d'un continuum de spéciation, et leur degré de divergence peut être appuyé par différents types de preuves correspondant aux concepts d'espèce proposés dans la littérature. Le concept morphologique, hérité de Linné (1758), repose sur la divergence phénotypique ; le concept biologique, défendu par Mayr (1999), sur l'isolement reproductif ; et le concept écologique, proposé par Van Valen (1976), sur l'occupation de niches distinctes. Les concepts génétique et phylogénétique, quant à eux, s'appuient respectivement sur la description de groupes génétiques différenciés (Baker & Hoofer, 2003) et sur la monophylie des lignées (Cracraft, 1983). Enfin, le concept de cohésion, illustré notamment par les travaux sur les loups et les coyotes (Rutledge et al., 2010), constitue une autre source d'argumentation pour évaluer la divergence entre lignées, correspondant à des groupes génétiques distincts conservant une cohésion malgré des flux de gènes. Dans cette thèse, le concept de continuum de spéciation ainsi que ces différents concepts d'espèce seront mobilisés pour discuter de la divergence entre l'érable à sucre et ses plus proches parents (*i.e.* cinq autres taxons d'érables de l'est de l'Amérique du Nord). Ces travaux constituent un préalable nécessaire à l'identification de l'adaptation génomique en lien avec l'adaptation au climat chez l'érable à sucre.

0.4 L'érable à sucre

0.4.1 Importance et menaces

L'érable à sucre est une espèce dominante des forêts tempérées de l'est de l'Amérique du Nord, distribuée entre les degrés 60° et 95° de longitude ouest, et 35° à 50° de latitude nord. Il occupe un rôle structurel majeur pour les forêts tempérées d'Amérique du Nord car il constitue l'espèce majoritaire de sept types de couverts forestiers décrits par la Société des Forestiers Américains. On le retrouve également dans 27 autres types de couverts forestiers ; 17 en tant qu'espèce commune associée et 10 autres en tant qu'espèce peu fréquente (Godman et al., 1990). Il joue un rôle crucial dans le maintien des processus écosystémiques et de la biodiversité en soutenant une diversité considérable d'invertébrés, dont divers insectes herbivores (Maguire et al., 2016). Son rôle dans le cycle de l'azote a également été mis en évidence dans le passé (Lovett & Mitchell, 2004). De plus, l'érable à sucre à un rôle économique important étant donné qu'il est exploité pour son bois d'œuvre de haute qualité et qu'il constitue la base de toute l'industrie du sirop d'érable (Chamberlain et al., 2019; Murphy et al., 2012). Cette dernière fonction, lui confère une importance socio-culturelle indéniable, car il est au centre d'activités économiques et sociales de nombreuses communautés humaines (Hinrichs, 1998). Cette espèce fournit également un feuillage automnal spectaculaire, qui est un moteur important du commerce touristique d'automne sur la côte Est de l'Amérique du Nord (Cook, 1991).

Cependant, les forêts d'érable à sucre sont confrontées à plusieurs menaces et défis dans le contexte actuel des changements globaux. En effet, l'érable à sucre est sensible à certaines espèces envahissantes comme l'érable de Norvège avec lequel il est en compétition (Paquette et al., 2012), le nerprun bourdaine qui a un effet négatif sur la croissance de ses semis (Hamelin et al., 2016), ou les vers de terre exotiques et le longicorne asiatique qui augmentent ses taux de mortalité (Bal et al., 2017; Dodds et al., 2013). Dans le contexte des changements climatiques, il est attendu que l'aire de répartition de l'érable à sucre se trouve limitée par des événements climatiques tels que les vagues de chaleur, les sécheresses et les vagues de froid (Putnam & Reich,

2017). De plus, l'acidification des sols, un facteur connexe aux changements climatiques, participe à un phénomène de déclin de l'érable à sucre observé à travers sa distribution (Horsley et al., 2002; Sullivan et al., 2013). Enfin, il est attendu que sa migration vers des latitudes ou des altitudes plus élevées soient limités par les conditions édaphiques caractéristiques de la forêt boréale (Collin et al., 2017, 2018).

0.4.2 Structure génétique

Les patrons de répartition de la diversité génétique au sein des populations d'érable à sucre ont été étudiés à différentes échelles et en utilisant différents types de marqueurs génétiques : allozymes (Ballal et al., 1994; Foré, Hickey, Guttman, et al., 1992; Foré, Hickey, Vankat, et al., 1992; Geburek, 1993; Perry & Knowles, 1989; Young, Merriam, et al., 1993; Young, Warwick, et al., 1993) ; ADN polymorphe amplifié aléatoirement (Diochon et al., 2003; Gunter et al., 2000) et microsatellites (Graignic et al., 2013, 2016, 2018a; Harmon et al., 2017; Khodwekar et al., 2015; Vargas-Rodriguez et al., 2015). Dans l'ensemble, l'érable à sucre présente une diversité génétique relativement élevée (Graignic et al., 2016) et la répartition de la variation génétique s'est avérée peu structurée (Diochon et al., 2003; Geburek, 1993; Graignic et al., 2018; Gunter et al., 2000b; Khodwekar et al., 2015; Perry & Knowles, 1989). Il s'agit d'un schéma fréquent pour les arbres dont le pollen et les graines sont dispersés par le vent, car ce mode de propagation implique des flux génétiques élevés entre les populations (Nybom, 2004). Néanmoins, comme explicité plus avant, plusieurs espèces forestières nord-américaines montrent des schémas de répartition de la diversité génétique illustrant les effets des oscillations glaciaires (Hewitt, 2004; Jaramillo-Correa et al., 2009). Malgré le consensus selon lequel les populations d'érables à sucre sont connectées par des flux génétiques participant à homogénéiser la répartition de la variation génétique, certaines études ont mis en évidence des patrons de répartition de la diversité génétique le long de gradients latitudinaux et longitudinaux (Vargas-Rodriguez et al., 2015; A.G. Young et al., 1993). Cette structure génétique a été interprétée dans un cas comme des traces des effets fondateurs subis par les populations lors de la recolonisation port-glaciaire (Vargas-Rodriguez et al., 2015), et dans l'autre cas comme un effet putatif de l'adaptation à l'environnement (A G Young et al.,

1993), en particulier la température et la composition du sol le long des gradients latitudinaux et les précipitations le long des gradients longitudinaux (Rowe, 1972).

0.4.3 Patrons d'adaptation locale issus des jardins communs

Cette dernière observation révèle un signal d'adaptation locale de certaines populations d'érable à sucre au sein de son aire de répartition. Des expériences récentes de jardins communs ont étudié l'effet de la provenance géographique sur la variation de traits phénotypiques chez des érables à sucre. L'adaptation génétique le long d'un gradient latitudinal a été suggérée après l'observation parmi diverses provenances testées de différentes stratégies de respiration (Gunderson et al., 2000), de date de début de germination (McCarragher et al., 2011) et de réponse du débourrement à la photopériode (Ren et al., 2021). De plus, Guo et al. (2020) ont observé différents moments de développement des feuilles selon les provenances et ont suggéré une adaptation locale aux températures printanières influencées par la proximité de la mer, évoquant à nouveau une adaptation génétique le long d'un gradient longitudinal. Par ailleurs, une étude plus ancienne réalisée par Ledig et Korbobo (1983) suggère une adaptation locale au sein des populations d'érable à sucre le long d'un gradient altitudinal basée sur l'observation de la variation de la réponse photosynthétique. Ces résultats peuvent être mis en parallèle avec ceux de Wallace et al. (2018), qui ont montré que les communautés microbiennes foliaires et racinaires diffèrent également selon l'altitude chez cette espèce. Or, plusieurs travaux récents ont démontré l'influence des microbiotes sur l'expression du phénotype de leurs hôtes (Henry et al., 2021; Téfit et al., 2023), ainsi que sur la réponse des plantes à la sécheresse (Cosme, 2023). Dans ce contexte, il est envisageable que la réponse plastique décrite par Ledig et Korbobo (1983) reflète en partie une interaction avec des communautés microbiennes spécifiques. D'autre part, selon certains jardins communs, la capacité d'adaptation de l'érable à sucre aux conditions climatiques pourrait reposer en grande partie sur la plasticité phénotypique plutôt que sur sa variation génétique (Burton et al. 1996; Gunderson, Norby et Wullschleger 2000; Guo et al. 2023). Enfin, les tests de germination et de transplantation d'érable à sucre dans des sols de forêts

boréales suggèrent une baisse des performances de l'érable à sucre dans des sols acides (Collin et al., 2017, 2018).

0.5 Objectifs et hypothèses de la thèse

L'objectif général de cette thèse est de documenter la variation génomique adaptative des populations d'érable à sucre du Québec face aux changements climatiques comme espèce modèle pour le devenir des forêts tempérées d'Amérique du Nord. Afin d'identifier correctement la diversité génétique en lien avec l'adaptation, il convient en premier lieu de documenter les relations existantes entre l'érable à sucre et les espèces qui lui sont proches. J'ai consacré les deux premiers chapitres de cette thèse à explorer cette problématique à différentes échelles de proximité génétique : (i) l'échelle du genre et de la série taxonomique pour le chapitre un (*i.e.* plus de deux taxons) et (ii) l'échelle de la délinéation génétique entre deux taxons collectés en sympatrie pour le chapitre deux. Le troisième chapitre quant à lui s'attèle à caractériser la diversité génétique des populations d'érables à sucre au Québec (Figure 0.2). Ce chapitre permet tout d'abord d'écarter les patrons de distribution de fréquences alléliques dues aux évènements démographiques passés, résultats des cycles glaciaires, pour discuter sereinement des signaux d'adaptation locale chez cette espèce. Enfin cette thèse s'inscrit dans le concept de continuum de spéciation et du concept d'espèce unifiée définit par De Queiroz (2007) car chacun de ces chapitres s'intéresse au degré de divergence, ainsi que les facteurs pouvant participer à cette divergence, entre des entités de différents niveaux taxonomiques correspondant à des étapes du processus de spéciation ; *i.e.* populations intraspécifiques, écotypes, sous-espèces, espèces.

Le premier chapitre a pour but de clarifier les relations phylogénétiques de l'érable à sucre avec les autres érables de la série taxonomique *Saccharodendron*. Ce chapitre cherche à répondre à la question suivante : est-ce que l'érable à sucre *sensu stricto* (*Acer saccharum* Marshall) forme une lignée évolutive distincte au sein de la série *Saccharodendron* ? Pour répondre à cette question j'ai reconstruit les relations phylogénétiques au sein du genre *Acer* en accentuant le nombre d'individus des taxons de cette série. En effet, bien que les phylogénies soient généralement

produites en incluant un ou deux individus par taxon, il est possible de discuter des relations entre individus au sein d'un même taxon en augmentant le nombre d'individus. Cette méthode relève de la phylogéographie et permet d'avoir une première idée de la structure génétique au sein des taxons, pouvant être influencée par la géographie ou leur histoire démographique (Avise, 2000). Dans le cas de ce chapitre l'augmentation de la résolution phylogéographique au sein la série taxonomique *Saccharodendron* permet de tester les hypothèses suivantes : i) les taxons de cette série sont en effet une seule et unique espèce extrêmement variable (Dansereau & Desmarais, 1947; de Jong, 2002; Desmarais, 1952; Kriebel, 1957) et ii) ces taxons sont génétiquement distincts (Areces-Berazain et al., 2021; Jackson et al., 2021). Selon la première hypothèse, certains taxons de cette série seraient polyphylétiques, c'est-à-dire que les individus attribués à un même taxon sur la base de critères morphologiques n'appartiendraient pas à une seule lignée phylogénétique. En revanche, selon la deuxième hypothèse, ils seraient réciproquement monophylétiques, signifiant que chaque taxon défini par des critères morphologiques regrouperait uniquement les individus issus d'un ancêtre commun exclusif.

Le deuxième chapitre porte une attention plus particulière à la délinéation génétique entre l'érable à sucre et l'érable noir dans des populations du Québec. En effet l'érable noir est considéré dans la littérature comme le plus proche parent de l'érable à sucre (Hilaire & Graves, 1999). De plus, l'érable noir est le seul taxon de la série *Saccharodendron* vivant en sympatrie avec l'érable à sucre sur une large partie de sa répartition (Gabriel, 1990). Par ailleurs, des cas d'hybridation forcée ont été reportés (Kriebel, 1989) et l'hybridation naturelle est supposée fréquente en raison de l'observation de phénotypes foliaires intermédiaires (Dansereau & Desmarais, 1947). De ce fait, il est possible que l'hybridation avec l'érable noir influence les fréquences alléliques dans des populations d'érable à sucre au Québec. Afin de prendre en compte cette éventualité pour la détection de la diversité génétique adaptative chez l'érable à sucre, le deuxième chapitre de cette thèse cherchait à répondre à cette question : est-ce que l'érable à sucre et l'érable noir sont génétiquement distincts quand ils sont présents en sympatrie ? Pour répondre à cette question j'ai étudié la structure génétique au sein de peuplements contenant des érables à sucre et d'érables noirs dans le Sud du Québec. Dans ce chapitre, les hypothèses de recherche étaient les suivantes : i) l'érable à sucre et l'érable noir sont deux formes

morphologiques de la même espèce génétique et ii) l'érable à sucre et l'érable noir sont constitués de groupes génétiques distincts même lorsqu'ils grandissent en sympatrie. Selon la première hypothèse, on s'attend à ce que les individus présentant des morphologies distinctes, soit d'érables à sucre soit d'érables noirs, ne soient pas discriminés par la variation génétique. Au contraire, selon la deuxième hypothèse on s'attend à ce que la distinction morphologique entre les deux érables corresponde à une distinction génétique.

Enfin, le troisième chapitre est consacré à la recherche de la diversité génétique adaptative chez l'érable à sucre. Les résultats des précédents chapitres ont permis de s'assurer que les fréquences alléliques des populations étudiées dans ce dernier chapitre ne sont pas influencées par de l'hybridation avec les espèces proches. Cependant, avant d'identifier la variation génétique en lien avec l'adaptation, il convient tout d'abord étudier la structure génétique des populations afin de mettre en évidence des patrons de distribution de la diversité génétique qui pourrait être due à des processus démographiques passés (Rellstab et al., 2015). Compte tenu de la littérature, j'émets les hypothèses suivantes : i) les populations du Québec ne sont pas structurées génétiquement (Graignic et al., 2018; Gunter et al., 2000; Perry & Knowles, 1989), ii) les populations du Québec présentent une structure génétique illustrant des lignées anciennes formées lors de la recolonisation post-glaciaire (Jaramillo-Correa et al., 2009). Sous la première hypothèse, une forte homogénéité génétique entre individus est attendue, se traduisant par une faible différenciation génétique au sein des populations d'érable à sucre au Québec. Sous la seconde hypothèse, je m'attends à identifier deux grands groupes génétiques séparés approximativement par la chaîne des Appalaches, correspondant aux lignées issues de routes de recolonisation distinctes post-glaciation. Dans un deuxième temps, des méthodes de génomique du paysage ont été mises à profit pour la détection de la variation génomique impliquée dans l'adaptation locale à l'échelle de la province, mais également à l'échelle locale parmi des paires de populations situées sur un gradient d'altitude. Concernant cet aspect, et compte tenu de la littérature, il est attendu que : i) les gradients latitudinaux et longitudinaux, de température et d'humidité respectivement, soient associés à la distribution de la variation génomique adaptative à l'échelle de la province (Guo et al., 2020; Rowe, 1972; A.G. Young et al., 1993), ii) le signal

d'adaptation soit plus faible à l'échelle locale qu'à l'échelle de la province en raison des effets contraignants de la migration sur la divergence sélective (Kremer et al., 2012).

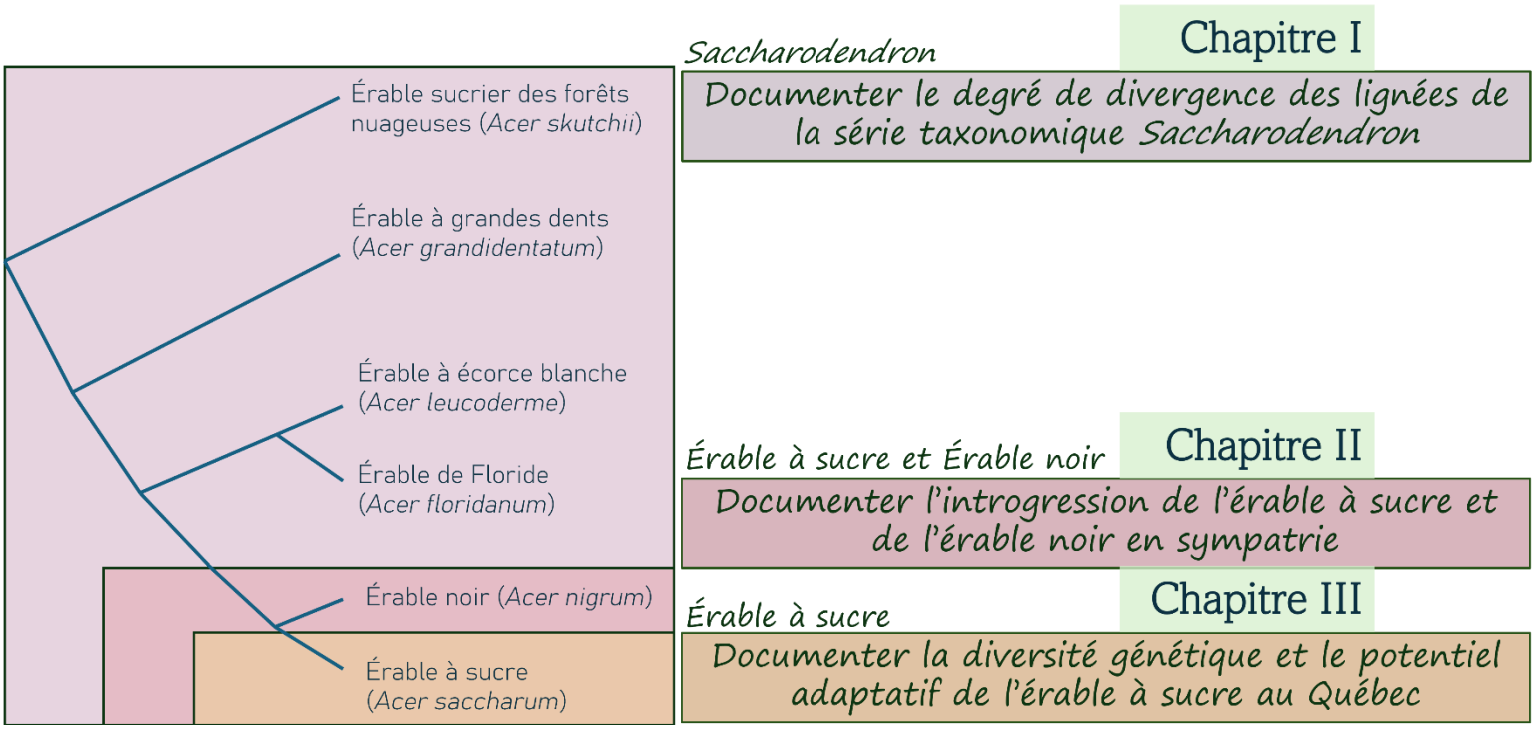

Figure 0.2 : Schéma conceptuel résumant le déroulé de la thèse et les objectifs de chaque chapitre

CHAPITRE 1 :

Combining genome skimming and assembly-alignment-free (AAF) phylogenetic reconstruction for detection of Evolutionarily Significant Units (ESUs) within genus *Acer*

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1.1 Abstract

In the context of the ongoing sixth biodiversity crisis, defining management units has become an urgent priority. Identifying reciprocal monophyly within taxonomic groups plays a key role in delineating evolutionarily significant units (ESUs) and can therefore support conservation efforts. Yet, detection of monophyly through phylogenomic studies requires expensive deep-coverage sequencing and computationally intensive bioinformatics workflows, including genome assembly and alignment-based phylogenetic reconstruction. In this study, we introduce a cost-effective and rapid alternative for assessing putative ESUs within genera. As a case study, we applied a genome skimming approach to sequence 46 *Acer* taxa and reconstructed their phylogenetic relationships using an assembly-alignment-free (AAF) method. Our first objective was to evaluate whether this approach could accurately recover clades that had been well supported in previous phylogenetic studies based on traditional markers and methods. Our second objective focused on the taxonomic series *Saccharodendron*, which includes sugar maple (*Acer saccharum*, Marshall) along with at least five other maple lineages for which no consensus nomenclature currently exists. By increasing taxon sampling within this group, we improved phylogenetic resolution among these closely related maples distributed across eastern North America. Ultimately, our method successfully recovered the backbone of the *Acer* phylogeny, as established by previous studies. Additionally, we provided evidence that the *Saccharodendron* lineages are reciprocally monophyletic, supporting their classification as ESUs. Since reciprocal monophyly is a key criterion in the phylogenetic species concept, we also discuss the possibility of recognizing these lineages as distinct species. Overall, our study demonstrates the effectiveness of the AAF method applied to genome skims for phylogenetic reconstruction and ESU delineation. Given its affordability and efficiency, this approach holds clear potential for conservation applications.

Keywords: systematics, assembly-alignment free phylogenetic, genome skimming, Acer genus, sugar maple

1.2 Introduction

Speciation is a dynamic and diversifying process involving different evolutionary mechanisms applying to populations, such as local adaptation and genetic isolation. Consequently, the speciation process creates a population-species continuum along which various levels of intraspecific diversity are documented by either ecological, morphological or genetic variation. Regarding conservation purposes, intraspecific diversity observed along populations-species continuum is taken into account through the definition of Evolutionarily Significant Units (ESUs) (Coates et al., 2018; Crandall et al., 2000; Moritz, 1994). After its original description by Ryder (1986) the concept of ESUs has been heatedly debated in the literature and several definitions were proposed (Fraser & Bernatchez, 2001). In this study, Evolutionarily Significant Units (ESUs) are defined as independently evolving genetic lineages, following a lineage-based ESU concept (Crandall et al., 2000; Waples, 1991). This definition of an ESU matches the concept of phylogenetic species as defined by Cracraft (1983) thus allowing the concept of ESU to be applied at both the intraspecific and specific levels. This definition allows to apprehend the value of clarifying phylogenetic relationships within a genus for conservation purposes. Indeed, besides its cooperation for the delineation of management units such as ESUs, phylogeny is useful for conservation biology as it allows to evaluate phylogenetic diversity and thus participates in conservation decisions for geographical areas (Coates, Byrne, and Moritz 2018). Moreover, phylogenetic diversity reflects the depth of evolutionary relationships, that is, the amount of evolutionary history contained within a group, and thus extends beyond taxonomic or functional diversity. In the context of the current erosion of biodiversity, incorporating this dimension into conservation strategies is particularly important to maximize the preservation of evolutionary heritage.

The dominant approach in phylogenetic inference from genomic data involves assembling and aligning DNA sequences, then using these alignments to infer homology between nucleotide positions. Traditionally, the assembly and alignment steps required high sequencing depth (Bohmann et al., 2020; Coissac et al., 2016). However, producing deep-

coverage data is expensive and the recently developed approach of genome skimming, that is shallow sequencing (0.1-10 depth) of random short reads across the genome (Straub et al., 2012), allowed to lower the minimum depth required for phylogenetic reconstruction. Indeed, this sequencing approach has been successfully used to recover high-copy regions of different genomes, including plastomes, mitochondrial DNA (mtDNA), nuclear ribosomal DNA (nrDNA) and some single copy nuclear genes (Dodsworth, 2015; Liu et al., 2021; Straub et al., 2012). While organelle genomes are easily recovered from genome skims and can be used for phylogenetic inference (Nauheimer et al., 2019; Richter et al., 2015), assembling or mapping nuclear genomes from this type of data is usually not feasible (Xu et al., 2022). The recovery of single copy nuclear genes usually necessitates a minimum of 10x coverage (Liu et al., 2021). Thus, genome skims have been mainly used for phylogenetic inference from organelle genomes and while these are valid and informative, they discard the phylogenetic signal contained in nuclear data, which represents the evolutionary history of any non-clonal organism (Bohmann et al., 2020). Consequently, the only way to harness genome skims for phylogenetic reconstruction without having to put aside nuclear data is to skip the assembly and alignment steps. Recently, several studies have proposed methodologies to exploit unassembled reads directly for phylogenetic inference (Fan et al., 2015; Sarmashghi et al., 2019). These methods rely on the distribution of short DNA fragments, known as k-mers, across individuals' genomes. In this context, *k* represents the length of the substring extracted from the original sequences. The probability of finding a sufficiently long common k-mer in two genomes is assumed to be directly related to their evolutionary distance (Röhling et al., 2020).

While combining genome skims with an assembly-alignment-free (AAF) method for phylogenetic reconstruction could lower sequencing costs as well as needs in computational power, this procedure has rarely been investigated (Xu et al., 2022). Here, we propose to harness this procedure for retrieving monophyletic lineages within *Acer* genus. The taxonomy of this genus has long been regarded as complex due to the significant morphological variation in vegetative traits and the species' tendency to

hybridize (de Jong, 1976; Grimm et al., 2007; Liao et al., 2010; Saeki et al., 2011). Over the years, several classification systems have been proposed as an attempt to organize the specific and infraspecific levels of the genus into sections and series (*i.e.* subgeneric taxonomic groups), mainly based on morphological characters such as leaves and flowering structures (de Jong, 2002, 1976; Momotani, 1962; Murray, 1970; van Gelderen et al., 1994). The well-used classification system of de Jong (2002) classifies 156 species and subspecies into 19 sections, six of which were subdivided into series. Over the last two decades, several studies have attempted to recover the monophyly of these infrageneric groups using various molecular data, focusing either on the relationships within specific sections (Fan et al., 2023; Grimm et al., 2007; Harris, Chen, et al., 2017; Li, 2011; Zhang et al., 2010) or on the entire genus by sampling as many sections as possible (Areces-Berazain et al., 2020, 2021; Fu et al., 2024; Gao et al., 2020; Grimm et al., 2006; Harris, Frawley, et al., 2017; Li et al., 2006, 2019; Wang et al., 2020). Ultimately, phylogenomic studies recovered most of the sections described by de Jong (2002) as monophyletic except for a few sections; *e.g*. sections *Acer* and *Lithocarpa* found polyphyletic in different phylogenetic studies (Areces-Berazain et al., 2021; Gao et al., 2020; Li et al., 2006, 2019; Wang et al., 2020). Overall, phylogenetic studies have contributed to resolving the backbone of relationships within the genus and identifying sections defined by de Jong (2002) that are problematic, *i.e.* for which monophyly is not consistently found across studies. This study aims to test the efficiency of the assembly-alignment-free (AAF) method for phylogenetic reconstruction applied on genome skims to retrieve the backbone of relationships within *Acer*.

Additionally, this study brings a particular focus on the series *Saccharodendron* within section *Acer*, as defined by de Jong (2002), regrouping the emblematic sugar maple (*Acer saccharum* Marshall) and its relatives. In the literature, the sugar maple is considered an extremely variable and polymorphic species, which thus declines in various subspecies or varieties across a wide geographical range (Godman et al., 1990). Therefore, several nomenclatures of this series has been proposed over the years and nowadays we can consider that it regroups seven to ten maples taxa distributed from Quebec to Guatemala

and designated either as species, subspecies or varieties (Davis, 2021; de Jong, 2002; Desmarais, 1952; Grimshaw & Bayton, 2010; Kriebel, 1957; Rehder, 1921; van Gelderen et al., 1994).

Ultimately, the series *Saccharodendron* makes a good representation of a population species continuum. Indeed, these taxa are supposed to be greatly related because they all have emerged recently from a common ancestor. The cloud forest sugar maple (*Acer skutchii* Rehder) is the southernmost taxon of this series since it is distributed in mountains of Mexico (Chiapas, Tamaulipas) and Guatemala. This maple is supposed to have diverged after Last Glacial Maximum (LGM) from relict populations of sugar maple that had extended its distribution southward because of the glacier (Vargas-Rodriguez & Platt, 2012). The bigtooth maple (*Acer grandidentatum* Desmarais) is the taxon of this series presenting the westernmost distribution. Given its disjunct distribution, this taxon has probably diverged because of geographical isolation (Areces-Berazain et al., 2021; Gao et al., 2020). The other taxa of this series are all distributed in the eastern parts of United States and south Canada. The causes of their divergence remain hypothetical today; it could result from either local adaptation to the environment (Desmarais, 1952; Kriebel, 1957) or isolation during the Quaternary glacial cycles (Jackson, 2020). The Florida maple (*Acer floridanum* Desmarais) and the chalk bark maple (*Acer leucoderme* Desmarais) are distributed in southern warm climates while the sugar maple *sensu stricto* (*A. saccharum*) and the black maple (*Acer nigrum* Desmarais) are distributed in the northern cooler climates. Depending on different authors, this series also includes other taxa, however our goal is not to make its taxonomical review but rather to pinpoint the great confusion around the taxonomical levels within this series. Indeed, phylogenetic studies on *Acer* genus including the taxa of series *Saccharodendron* often refer to them as subspecies (Gao et al., 2020; Grimm et al., 2006, 2007; Renner et al., 2008) with two exceptions (Areces-Berazain et al., 2021; Li et al., 2019). In their phylogenomic reconstruction of genus *Acer*, Areces-Berazain, Hinsinger, and Strijk (2021) brought evidence for distinct evolutionary lineages for *A. skutchii* and *A. grandidentatum*. However, their study included one sample per taxon which is insufficient for clearly

delineating evolutionary lineages among closely related taxa along the population-species continuum (Avise et al., 1987). Moreover, a recent study on population genetic among these taxa brought evidence of distinct genetic pools, thus suggesting they could be considered as species (Jackson et al., 2021). Ultimately, beyond the initial goal of retrieving the backbone of *Acer* phylogeny with a new method we further investigated relationships within the *Saccharodendron* series by rising the number of individuals for its taxa.

Beyond the taxonomical deliberation of the position of these taxa along the population-species continuum, there is a conservation concern for five of them. Indeed, Nature Serve Explorer (NatureServe, 2024) records; i) *A. floridanum* as vulnerable in Florida, ii) *A. leucoderme* as vulnerable in Tennessee and North Carolina, imperiled in Arkansas and Louisiana and seriously imperiled in Mississippi, iii) *A. grandidentatum* as vulnerable in New Mexico and Wyoming, and seriously imperiled in Colorado and Nevada, iv) *A. nigrum* as vulnerable in Quebec, Vermont, Massachusetts and Georgia, imperiled in New Hampshire and seriously imperiled in New Jersey, North Carolina, Arkansas and Kansas. Moreover, *A. skutchii* is listed as critically endangered by the IUCN red list (IUCN Red List of Threatened Species, 2017). Consequently, it might be valuable to focus on delineating ESUs among these North American maple taxa to further discuss their conservation needs.

In this study, we propose a novel phylogeny of the genus *Acer* based on genome skims and inferred by a AAF method using k-mers counts (Fan et al., 2015). According to literature, the AAF method designed by Fan et al. (2015) performed well with other genera as it managed to recover inter- and intra-specific relationships among six species of *Quercus* as well as divergences among 17 genera of the *Phrynosomatidae* subfamily (Fan et al., 2018). However, this method has never been evaluated on genus *Acer*, as well as there is yet no phylogeny recovered from genome skims for this genus. The first goal of this study is to evaluate the performance of the association of genome skimming with AAF method for retrieving the backbone topology of phylogenetic relationships within genus *Acer*. In this regard, our hypotheses of monophyly are based on sections defined

by de Jong (2002), with the adjustment proposed by Harris et al. (2017) to regroup sections *Hyptiocarpa* and *Rubra*. According to literature we expect to retrieve most sections defined by de Jong (2002) and Harris (2017) as monophyletic except for sections *Trifoliata*, *Pentaphylla*, *Acer*, and *Lithocarpa* (Areces-Berazain et al., 2021; Li et al., 2019). Our second goal is to delineate ESUs within the series *Saccharodendron*. To this regard, we formulate two hypotheses: i) taxa of this series are indeed one and only extremely variable species (Dansereau & Desmarais, 1947; de Jong, 2002; Desmarais, 1952; Kriebel, 1957) and ii) taxa of this series display distinct gene pools suggesting species delineation (Areces-Berazain et al., 2021; Jackson et al., 2021). Under the first assumption we expect some of the taxa of this series to be polyphyletic while under the second hypothesis we expect them to be reciprocally monophyletic.

1.3 Material & Methods

1.3.1 Taxon sampling

We obtained leaf samples of 70 individuals belonging to 46 taxa of *Acer*, representing 15 out of the 18 currently validated sections (de Jong, 2002; Harris, Chen, et al., 2017), as well as two outgroup species: *Aesculus glabra* Willd. (1809) and *Dipteronia dyeriana* Henry (1903). Taxa of *Saccharodendron* series were more represented, up to seven individuals for *A. saccharum* and *A. nigrum*, to increase resolution of phylogenetic relationships within this series. Samples were either collected from living plants in natural populations, botanical gardens and arboretum or on dried herbarium samples (supplementary Table S1 in GitHub repository). When collected on living plants, leaves where dried at once with silica gel desiccant.

1.3.2 DNA extraction, library construction and sequencing

Total genomic DNA was extracted from 50 mg of dried leaves using QIAGEN Plant Mini Kit extraction (Qiagen, Germantown, MD) following manufacturer's instructions. Individual libraries (N=70) were prepared for Illumina sequencing using the NEBNext® Ultra™ II FS DNA Library Prep Kit (E7805, E6177) following the provided protocol for genomic inputs ≥100 ng. The only modification made to the latter protocol was to execute the ligated fragments size selection step as described in the protocol for genomic input ≤ 100 ng. Specific pairs of Illumina NEBNext primers were used as individual library barcodes. The libraries were pooled in equimolar amounts, with each contributing 160 ng to the final pool, which was then purified using SPRIselect beads (Beckman & Coulter) at a concentration of 0.9X (where X is the volume of the final pool). Sequencing of purified pool and demultiplexing was performed at The Centre d'expertise et de services Génome Québec (Montreal, QC, Canada) using one NovaSeq6000 sequencing lane.

1.3.3 Phylogenomic reconstruction

All scripts used during bio-informatic procedures can be consulted in this GitHub repository.

1.3.3.1 Preparation of merged sequences for each sample

Raw reads per individuals were analysed in FASTQC_0.11.9 for computation of sequencing statistics (Andrews, 2012). Individuals' statistic reports were synthetized by MultiQC_1.21 (Ewels et al. 2016). Raw paired-end reads were filtered based on statistical outputs; reads with an average minimum quality below Q20 were removed, along with duplicated and overrepresented sequences in fastp_0.23.0 (Chen et al., 2018). The individual filtered paired reads were then merged into sequences using the same software.

1.3.3.2 K-mer counting and k-mer length optimisation.

Individual merged sequences served as input for k-mer counting for 2<k<31 in jellyfish_2.3.0 (Marçais & Kingsford, 2011). We followed protocol described in Fan et al. (2015) to estimate optimal length of k-mers and phylogenomic reconstruction. Choosing the k value involves balancing k-mer homoplasy, which is more common with shorter k-mers, against the risk of mutations within the same k-mer, which is more likely with longer k-mers. Fan et al. (2015) based their choice of best k value on the theoretical predictions of the proportion of shared k-mers, *ph*, as it allowed them to choose k-mer length leading to accurate phylogeny reconstruction. First, calculation of *ph* necessitates GC content, total base pair and mean read length for each sample that were computed by the *ad hoc* python script (*gc.py*) provided by Fan et al. (2015). Second, calculation of *ph* uses counts of unique k-mers, as well as total number of k-mers and k-mers diversity (*i.e.* k-mers that appear at least one time, also called distinct k-mers). These k-mer counts were computed by an *ad hoc* python script following method described in a GitHub post (Clavijo, 2018). All following steps were undertaken in R_4.3.1 (R Core Team, 2022) following the *ad hoc* script (*phVSk.R*) provided by Fan et al. (2015) that we modified so that it fits our data. From k-mer counts, total base pair counts and mean read length, we estimated k-mer coverage (c), base coverage (bc) and genome size (gs) following formulas provided by Fan et al. (2015) [c = total number of k-mers / k-mer diversity; bc = c * mean read length / (mean read length - *k*-mer size + 1); gs = total base pair count / base coverage]. Calculation of *ph* for 12<k<31 was then undertaken using: (i) GC content for each sample, (ii) mean genome size and base coverage per species across k values, (iii) frequency distribution of k-mers and (iv) the default value for genetic distance among samples (d=0.1). Plotting *ph* values against k values in R_4.3.1 allowed to show k values for which *ph* estimation stabilizes across all samples. Thereafter, for each of these selected k values, we computed a frequency matrix of shared k-mers across samples using the *ad hoc* python script (*aaf_phylokmer.py*; Fan et al. 2015). The matrix of shared k-mers served as input for calculation of a distance matrix among samples and estimation of the phylogenetic relationship using the program fitch in the package PHYLIP (Künsch, 1989) implemented

in the *ad hoc* python script (*aaf_distance.py*; Fan et al. 2015). Given the low base coverage of samples for selected k values (supplementary Table S2), singletons (*i.e.* k-mers occurring just once) were not filtered out from the data to perform the phylogenetic reconstruction (Fan et al., 2015). Plotting and comparison of phylogenetic trees obtained for each k value was undertaken using ape_5.8 package (Paradis & Schliep, 2019) in R_4.3.1. We choose the optimal k value for k-mer length as the one from which phylogenetic relationships remain stables.

1.3.3.3 Construction of chronogram with bootstrap values

Fan et al. (2015) implemented a two-stage parametric bootstrap to assess node support in their AAF method for phylogenetic reconstruction. In the first stage, uncertainty due to incomplete genome coverage and sequencing error is modeled by adding normally distributed random noise to each pairwise genetic distance, with variance derived from analytical estimates and scaled conservatively to avoid inflating node support. Bootstrap trees are generated by repeatedly reconstructing phylogenies from these perturbed distance matrices. In the second stage, additional variation due to the stochastic accumulation of mutations is incorporated by further perturbing the distance matrix, accounting for covariances among distances arising from shared evolutionary history and overlapping k-mers. Node support is then estimated as the proportion of bootstrap trees recovering each branch.

Parametric bootstrap was performed by the *ad hoc* R script provided by Fan et al. (2015); phylogenetic relationships were recalculated for 100 resampling of the shared k-mer table computed for the 70 individuals and for the optimal length (k) of k-mers. Eventually, the final phylogenetic tree was plotted and fitted to a chronogram inferred by a relaxed molecular clock calibrated on age of the crown node found by Gao et al. (2020) and Areces-Berazain et al. (2021) (*i.e.* crown node estimated between 59 Ma and 61 Ma) using ape_5.8 in R_4.3.1.

1.4 Results

1.4.1 Sequencing results

On average, we recovered 44.5 x 10^6 raw reads per sample. After filtering and merging paired sequences, number of sequences available for analyses was 27.8 x 10^6 sequences per sample on average with a mean read length of 111.8 bp (supplementary Table S2).

1.4.2 k-mers statistics

No unique k-mers were found for k<12, thus estimation of the theoretical predictions of the proportion of shared k-mers, *ph*, for each sample was undertaken for 12<k<31. We observed that *ph* for all samples stabilized for k ≥ 19 and that the difference between estimations of *ph* and the hypothetical case if there were no k-mer homoplasy continued to decrease with larger k (Figure 1.1).

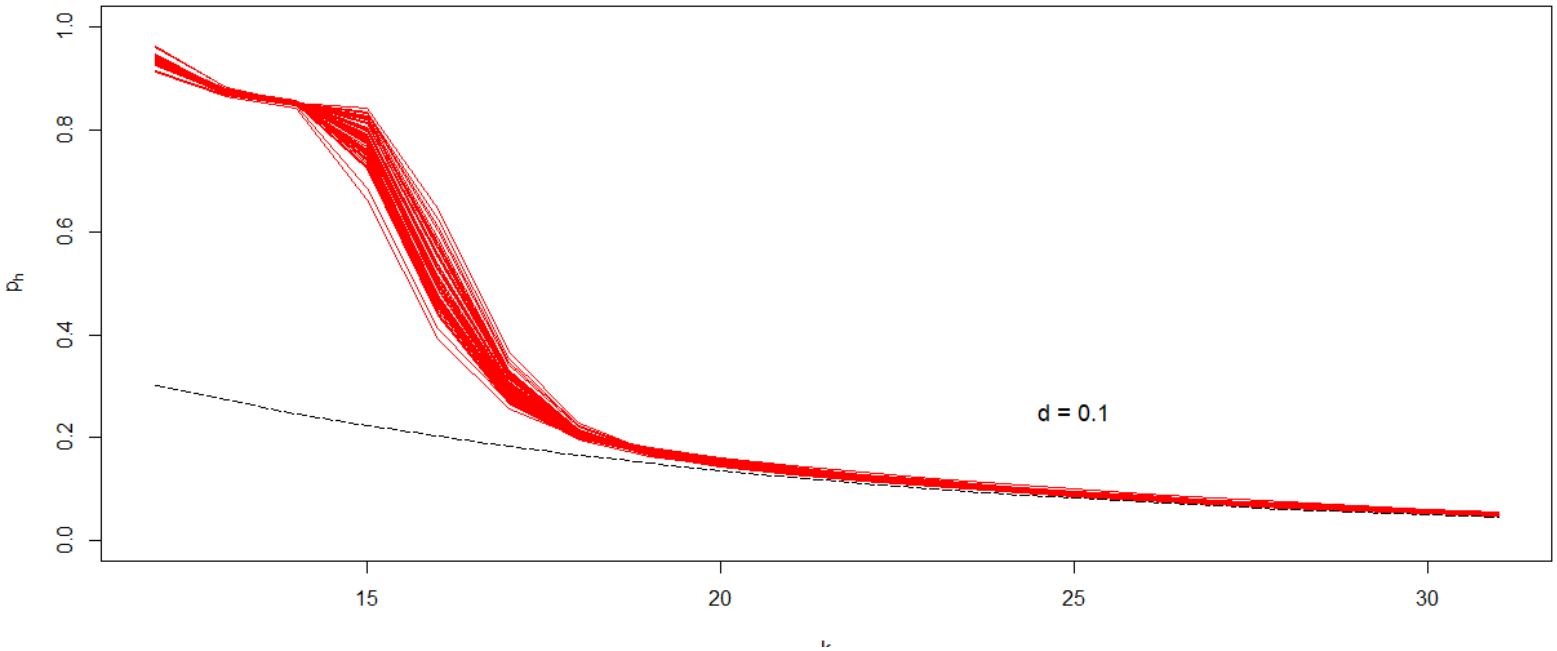

Figure 1.1: Theoretical predictions of the proportion of shared k-mers, ph, calculated from the observed frequency distribution of k-mers for the maple species (red lines) assuming the true distance between taxa is d = 0.1. The black dashed line stands for the hypothetical case where there is no k-mer homoplasy.

Therefore, phylogenetic relationships were investigated for 19<k<31. We observed that tree topology for backbone relationships among *Acer* genus stabilizes for k=24. Indeed, from k=24, the only differences in tree topologies estimated from various k-mers length distance matrices regarded recent divergences: phylogenetic tree based on 26-mers proposes other relationships among individuals within *A. floridanum* while phylogenetic reconstruction based on 29-mers suggests other relationships within individuals of *A. leucoderme* and *A. nigrum* (Supplementary Figure 1.4). Given that backbone relationships appear to be resolved for k=24, we chose this value of k as the optimal value for phylogenetic reconstruction and considered changes in tree topologies for k=26 and k=29 to reflect phylogeographic variability within *A. floridanum*, *A. leucoderme* and *A. nigrum*. From the 24-mers, we estimated the average base coverage to be 4.3x (supplementary Table

S4; mean = 4.30x, min = 2.45x, max = 6.84x, s.e. = 0.62x). The phylogenetic relationships, based on the shared 24-mers matrix, are presented as a chronogram in Figure 1.2, along with the bootstrap values for the nodes.

1.4.3 Phylogenetic relationships

Phylogenetic tree produced by AAF method displays two major clades with high bootstrap value (100); the first, composed by sections *Arguta*, *Negundo*, *Spicata* and *Palmata*, and the second, regrouping the other sections. Ultimately, all divergences are well supported with bootstrap value of 100, except for one node within *A. saccharum* individuals and three nodes within *A. floridanum* individuals (Figure 1.2). Sections *Acer*, *Lithocarpa*, *Palmata*, *Pentaphylla* and *Platanoidea,* as defined by de Jong (2002), are not monophyletic. All the taxa of the series *Saccharodendron* are reciprocally monophyletic except for *A. leucoderme* and *A. skutchii*, the latter being nested within the former.

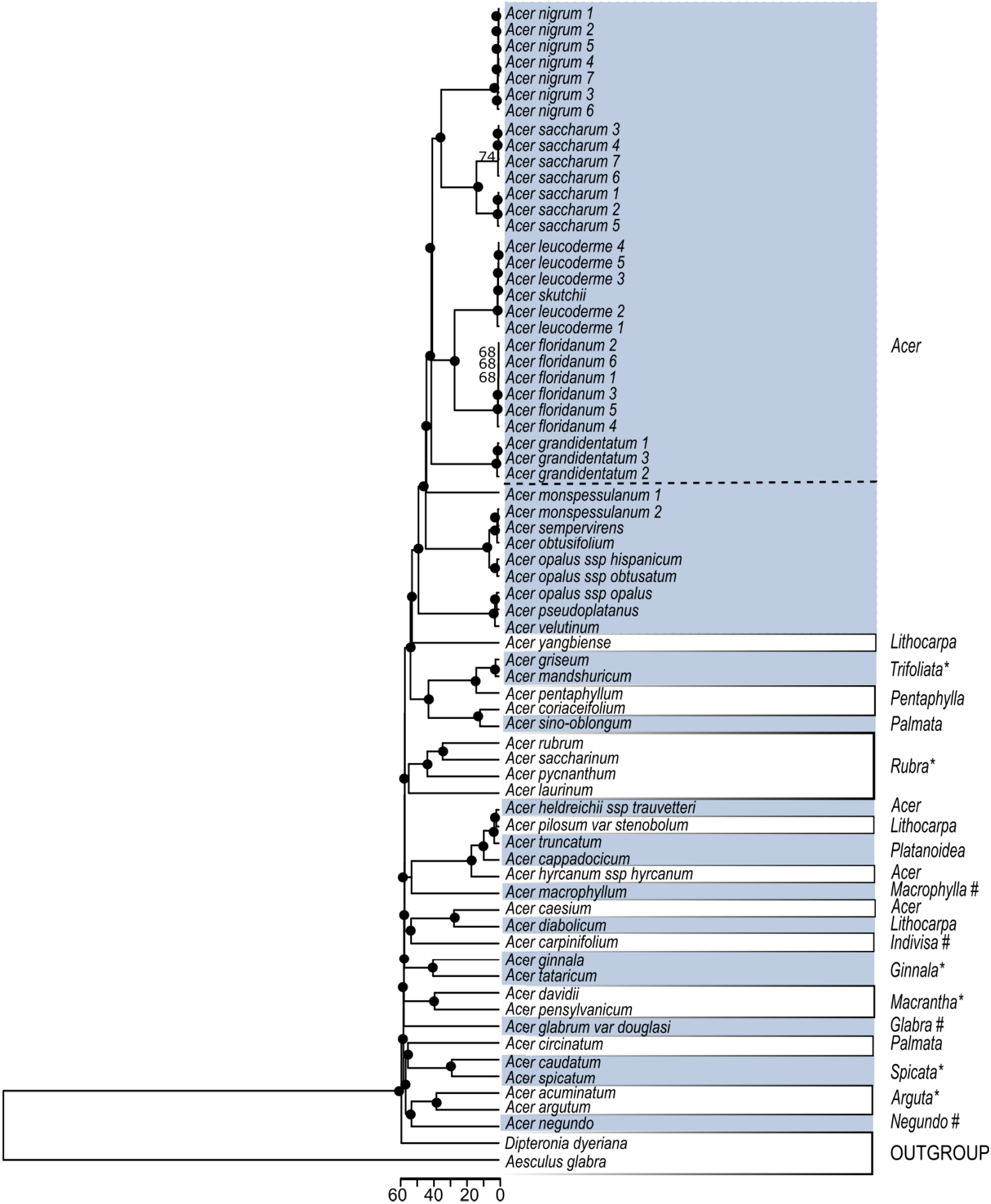
Acer nigrum 1
Acer nigrum 2
Acer nigrum 5
Acer nigrum 4
Acer nigrum 7
Acer nigrum 3
Acer nigrum 6
Acer saccharum 3
Acer saccharum 4
74
Acer saccharum 7
Acer saccharum 6
Acer saccharum 1
Acer saccharum 2
Acer saccharum 5
Acer leucoderme 4
Acer leucoderme 5
Acer leucoderme 3
Acer skutchii
Acer leucoderme 2
Acer leucoderme 1
Acer floridanum 2
68
Acer floridanum 6
68
Acer floridanum 1
68
Acer floridanum 3
Acer floridanum 5
Acer floridanum 4
Acer grandidentatum 1
Acer grandidentatum 3
Acer grandidentatum 2
Acer
Acer monspessulanum 1
Acer monspessulanum 2
Acer sempervirens
Acer obtusifolium
Acer opalus ssp hispanicum
Acer opalus ssp obtusatum
Acer opalus ssp opalus
Acer pseudoplatanus
Acer velutinum
Acer yangbiense
Lithocarpa
Acer griseum
Acer mandshuricum
Trifoliata*
Acer pentaphyllum
Acer coriaceifolium
Pentaphylla
Acer sino-oblongum
Palmata
Acer rubrum
Acer saccharinum
Acer pycnanthum
Acer laurinum
Rubra*
Acer heldreichii ssp trauvetteri
Acer
Acer pilosum var stenobolum
Lithocarpa
Acer truncatum
Acer cappadocicum
Platanoidea
Acer hyrcanum ssp hyrcanum
Acer
Acer macrophyllum
Macrophylla #
Acer caesium
Acer
Acer diabolicum
Lithocarpa
Acer carpinifolium
Indivisa #
Acer ginnala
Acer tataricum
Ginnala*
Acer davidii
Acer pensylvanicum
Macrantha*
Acer glabrum var douglasi
Glabra #
Acer circinatum
Palmata
Acer caudatum
Acer spicatum
Spicata*
Acer acuminatum
Acer argutum
Arguta*
Acer negundo
Negundo #
Dipteronia dyeriana
Aesculus glabra
OUTGROUP
60
40
20
0

Figure 1.2: Chronogram of phylogenetic relationships recovered by the AAF method on 70 genome skims of genus *Acer*. Time scale in Ma was calibrated using a relaxed molecular clock based on results of Areces-Berazain, Hinsinger, and Strijk (2021) and Gao et al. (2020). Sections as defined by de Jong (2002) and Harris (2017) are added beside species. Monophyletic sections are marked by * (or # when monotypic). The dashed line delimitates the taxa composing the series *Saccharodendron*. Nodes with bootstrap = 100 are marked by ● and when bootstrap < 100 the value is added beside the concerned node.

46 species of *Acer* with an increased sampling effort for taxa of the series *Saccharodendron* (section *Acer*). Ultimately, the AAF method recovered monophyly for ten well supported sections described by de Jong (2002) and Harris (2017) while problematic sections showing conflicting results in the literature remained unclear. Meanwhile, taxa of the series *Saccharodendron* display distinct evolutionary lineages except for *A. skutchii* that is nested within *A. leucoderme*. Our results suggest that the application of the AAF method on genome skims is an efficient procedure for ESUs' recovery and that taxa within the series *Saccharodendron* correspond to ESUs.

1.5.1 Phylogenetic relationships

Regarding phylogenetic relationships within *Acer* genus, the AAF method recovered a well supported divergence of one clade regrouping sections *Negundo, Arguta, Spicata, Palmata* and a second clade composed of the other sections (Areces-Berazain et al., 2021; Fu et al., 2024; Grimm et al., 2006; Li et al., 2019). Furthermore, we retrieved monophyly for height sections as described by de Jong (2002) which monophyly are also well-supported in the literature; *Arguta, Ginnala, Glabra, Indivisa, Macrantha, Macrophylla, Negundo, Spicata* (Areces-Berazain et al., 2021; Gao et al., 2020; Li et al., 2019). Moreover, the recent modification of section *Rubra* as defined by Harris et al. (2017), *i.e*. including previous section *Hyptiocarpa* (*Acer laurinum*), is well supported by our phylogeny as it has been in the literature (Areces-Berazain et al., 2021; Gao et al., 2020). Finally, our phylogeny supports the relationships within section *Acer*, between the North American taxa of series *Saccharodendron* and the European taxa of series *Monspessulana*, sharing a common Asian ancestor, implying that *A. saccharum* probably colonized North America

through a dispersal route from Europe passing by North Atlantic Land bridges (Areces-Berazain et al., 2021; Gao et al., 2020).

The AAF method also retrieved monophyly for section *Trifoliata* whereas this section was found polyphyletic by Li et al. (2019) and Areces-Berazain et al. (2021). However, these latter studies included two more species in this section which could have influenced lineage sorting. On the contrary, we recovered the sections *Palmata* and *Platanoidea* as polyphyletic whereas they constitute well supported monophylies in the literature (Areces-Berazain et al., 2021; Gao et al., 2020; Grimm et al., 2006; Li et al., 2019; Wang et al., 2020). First, in our study section *Palmata* is polyphyletic because *A. sino-oblogum* (section *Palmata*) is found sister to *A. coriaceifolium* (section *Pentaphylla*). It seems that the phylogenetic relationships within the *Palmata* section (*i.e.* between *A. sino-oblongum* and *A. circinatum*) were difficult to clarify in the context of our sampling. Indeed, in our study the *Palmata* section is represented by two species with very distinct distributions and morphologies; *A. sino-oblongum* has non-lobed leaves and is distributed in South China whereas *A. circinatum* has 7-11-lobed leaves and is distributed in western North America. According to the nomenclature of de Jong (2002) these two species are in different series within the *Palmata* section. Furthermore, *A. coriaceifolium* (section *Pentaphyllum*) has a leaf morphology and geographical distribution like that of *A. sino-oblongum* (section *Palmata*). Consequently, the phylogenetic association of *A. sino-oblongum* with *A. coriaceifolium* in our study may have been influenced by these similarities in distribution and morphologies. There is also a possibility that a sampling error was made and that these two species were confused. Finally, given that phylogenetic reconstructions are based on distance matrices calculated from shared k-mer frequencies, it is possible that some of the inconsistencies observed in sections *Palmata* and *Platanoidea* are related to this parameter.

This difficulty in discerning lineages within variable sections like *Palmata* could have been alleviated by adding more species from that section. For example, the phylogenomic studies which found a monophyletic lineage section *Palmata* had considered 10 to 25

species (Areces-Berazain et al., 2021; Gao et al., 2020; Li et al., 2019). However, Wang, Chen, and Zhang (2020) also found *A. sino-oblongum* and *A. coriaceifolium* as sisters in their phylogenetic reconstruction based on plastomes. This suggests that our data for these two species, through the stochasticity of genome skimming sequencing, are perhaps more composed of sequences originating from the plastome. This can greatly influence the results and, indeed, the studies that resolved the monophyly of section *Palmata* were based either on nuclear markers or combined matrices of nuclear and chloroplast data. Finally, while section *Platanoidea* is found polyphyletic here while it is usually monophyletic in other studies (Areces-Berazain et al., 2021; Dong et al., 2021; Gao et al., 2020; Li et al., 2019; Suh et al., 2000; Yu et al., 2022), it is found sister to section *Macrophylla* which is consistent with previous phylogenies (Areces-Berazain et al., 2021; Gao et al., 2020; Li et al., 2019).

Besides, we found that sections *Acer* and *Lithocarpa* display strong patterns of polyphyly that have already been described in literature (Areces-Berazain et al., 2021; Gao et al., 2020; Li et al., 2006, 2019; Wang et al., 2020; Yu et al., 2022). In our study we retrieve three associations of sections *Acer* and *Lithocarpa*; i) *A. caesium* (section *Acer*) is sister to *A. diabolicum* (section *Lithocarpa*), ii) *A. heldreichii ssp trauvetteri* (section *Acer*) is sister to *A. pilosum var stenobolum* (section *Lithocarpa*), iii) *A. yangbiense* (section *Lithocarpa*) is regrouped with the other taxa of section *Acer*. Furthermore, relationships within section *Acer* are not fully retrieved by the AAF method since species constituting the series *Monspessulana* within section *Acer* are not regrouping in a clade. In the literature, this series is either found polyphyletic (Areces-Berazain et al., 2021; Grimm et al., 2006, 2007; Renner et al., 2008) or monophyletic (Gao et al., 2020; Li et al., 2019). Moreover, we brought evidence of the polyphyly of section *Pentaphylla* as it had been documented before (Areces-Berazain et al., 2021; Gao et al., 2020; Li et al., 2006, 2019; Yu et al., 2022). We also find the phylogenetic association between sections *Pentaphylla* and *Trifoliata* as described by the latter previous studies.

Ultimately, the phylogenetic tree that we recovered by the AAF method based on genome skims shows similitudes with others phylogenetic trees obtained in the past, regarding either resolved or unresolved relationships. Especially, considering that our phylogeny is based on skimming data holding signals of both organelle and nuclear genomes, it makes sense that our results are similar than those of Areces-Berazain, Hinsinger, and Strijk (2021) that worked on a genome-wide supermatrix combining plastomes and nuclear sequences.

1.5.2 The Saccharodendron series

The current consensus topology identifies *A. grandidentatum* and *A. skutchii* as distinct clades that diverged earlier in *Saccharodendron*'s evolutionary history (Areces-Berazain et al., 2021). Additionally, it features a clade containing two sub-clades: one comprising *A. floridanum* and *A. leucoderme*, and the other including *A. nigrum* and *A. saccharum* (Areces-Berazain et al., 2021; Grimm et al., 2007). The phylogeny produced in our study matches this topology (Figure 1.2) except for the placement of the *A. skutchii* individual, originating from an herbarium specimen collected in Tamaulipas (Mexico), that grouped with *A. leucoderme* individuals from the southeastern USA. This clustering may reflect the genetic proximity reported by Vargas-Rodriguez et al. (2015). This past study based on microsatellites described signals of past connectivity and lack genetic differentiation between *A. saccharum* populations in the southeastern USA and a population of *A. skutchii* of eastern Mexico (Tamaulipas). However, definitive conclusions are challenging to make, as *A. leucoderme* populations were not included in that earlier study. Additionally, it is important to note that our analysis included only one individual of *A. skutchii*, compared to at least three individuals for the other members of *Saccharadendron*. This limited sampling likely influenced lineage sorting, underscoring the need for further phylogeographic investigations with more *A. skutchii* individuals to confidently clarify the evolutionary history of this lineage.

Our study further investigate phylogenetic relationships within the clade comprising *A. floridanum*, *A. leucoderme*, *A. nigrum*, and *A. saccharum*, as previous studies included less taxa of this series and only one individual per taxon (Areces-Berazain et al., 2021). Our findings indicate that the divergence between *A. nigrum* and *A. saccharum* predates the split between *A. floridanum* and *A. leucoderme* (Figure 1.2), a conclusion not previously reported. Additionally, *A. saccharum* individuals form two distinct lineages corresponding to the most recent divergence within the series. However, this divergence does not appear to be driven by geographic distance, as individuals from distant locations are closely related, while those from adjacent areas do not group together (Figure 1.3). This pattern might reflect the past isolation of populations that followed distinct postglacial migration routes (Graignic et al., 2018). In contrast, no sub-clades were observed within the lineages of *A. floridanum*, *A. leucoderme*, or *A. nigrum*. However, in *A. leucoderme*, geographically proximate individuals are more closely related phylogenetically (Figure 1.3). The stair-step topology observed within this lineage suggests that phylogeographic patterns are linked to colonization events. Conversely, the phylogenetic grouping within *A. floridanum* and *A. nigrum* does not correspond to their geographic origins (Figure 1.3), suggesting that geography does not significantly influence the genetic structure of these taxa. However, these interpretations should be further validated using traditional population genetics approaches. Moreover, Bayesian approaches such as DIYABC Random Forest could provide a more detailed insight into the evolutionary history of this taxonomic series.

Ultimately, regarding our second objective, it appears that taxa of the *Saccharodendron* series correspond to well supported reciprocal monophylies and thus can be considered as ESUs. They constitute distinct evolutionary lineages which supports the conclusions of Jackson et al. (2021) who highlighted distinct gene pools for these taxa based on microsatellite data. Furthermore, we interpret this result as evidence that these taxa keep genetic integrity along their range even if they are sympatric with other taxa of the series; *A. floridanum* is sympatric with *A. leucoderme*, and *A. nigrum* is sympatric with *A. saccharum*.

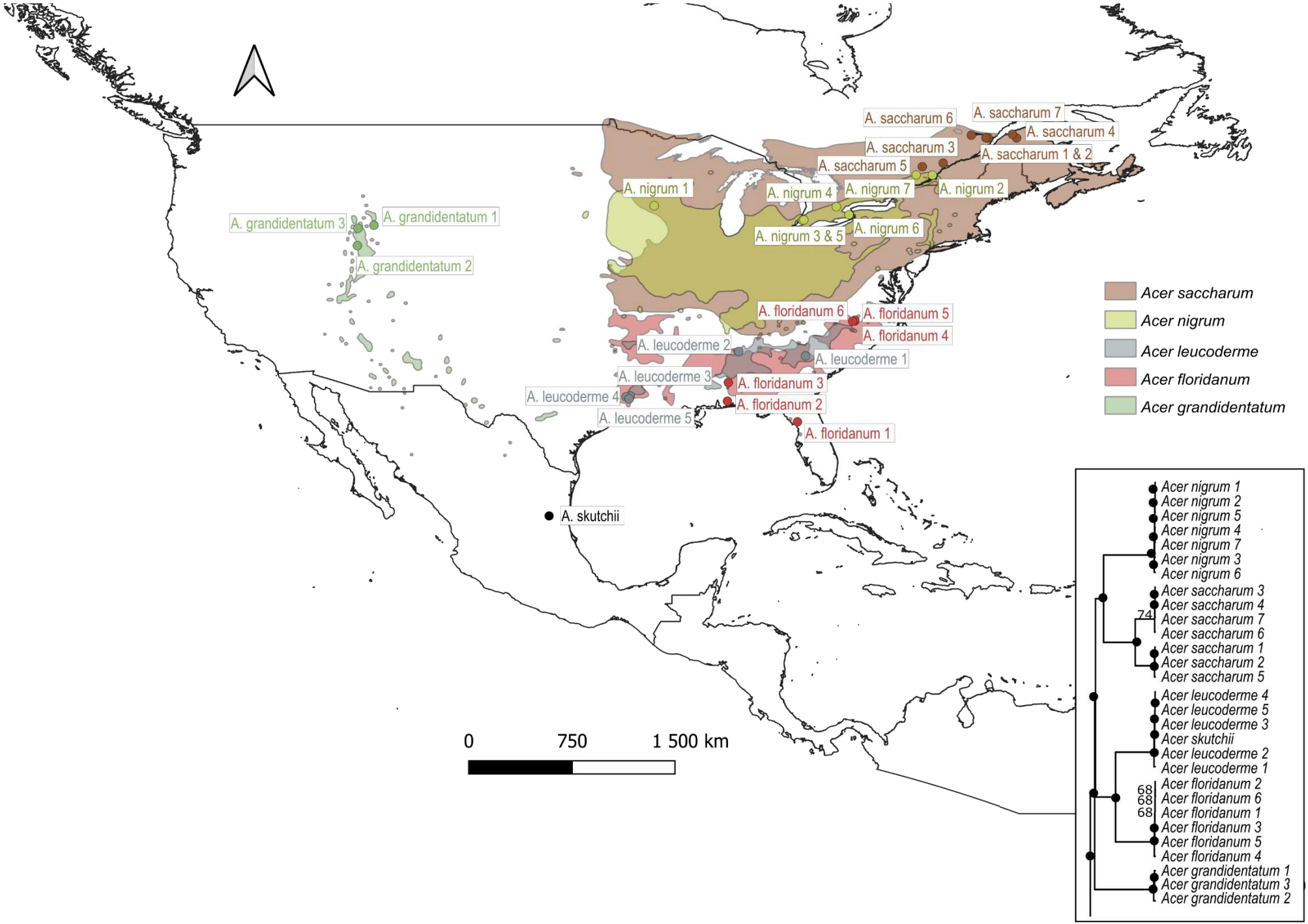

Figure 1.3: Distribution map and phylogenetic relationships of samples of taxa of the series *Saccharodendron*.

Especially, we argue that it should take part to reconsider taxonomical statuses of the sugar maple (*A. saccharum*) and the black maple (*A. nigrum*). Their consideration as subspecies in part of the literature (Areces-Berazain et al., 2021; de Jong, 2002; Gao et al., 2020; Hauer et al., 2021; Hilaire & Graves, 1999) is challenged as they respond to the phylogenetic concept of species and as they are sympatric for a large portion of their range. Indeed, these maples do not fit the traditional definition of subspecies, used by both botanists and zoologists, as "phenotypically distinct, allopatric sets of populations that may intergrade into each other at geographic boundaries" (Grant, 1981; Mayr, 1999). Nevertheless, we emphasize that Bayesian methods, such as Bayes Factor Delimitation (BFD) implemented in BEAST2, could further clarify the delimitation of ESUs within *Saccharodendron*.

1.6 Conclusion

In conclusion, the AAF method applied on genome skims efficiently recovered the backbone of the phylogenetic relationships among sections of genus *Acer*. Well supported divergences among sections were retrieved but unclear relationships among some sections remained unresolved. This first conclusion highlights the potential of a quicker and cheaper method for phylogenomic reconstruction. Otherwise, almost all lineages within *Saccharodendron* were clearly discriminated, supporting the hypothesis of distinct gene pools (Jackson et al., 2021).

These taxa were also reciprocally monophyletic responding to the definition of an ESU and the definition of a species according to the phylogenetic concept. Given the recent evolutionary history of the series *Saccharodendron* and the putative extensive hybridization between its taxa, they have been placed closer to the population-end of population-species continuum, thus interpreted as subspecies or ecotypes or varieties (Dansereau & Desmarais, 1947; Desmarais, 1952). Our result, combined with other recent studies, suggest that these taxa might be closer of the species-end of the population-species continuum, which could have repercussion on their conservation statuses. However, further investigation of their genetic structure, patterns of introgression, evolutive and demographic history are needed to draw confident conclusions.

Ultimately, besides confirming the efficiency of the AAF method for reconstructing phylogenetic relationships with low coverage data of non-model species, our results highlight the potential of this method for investigation of ESUs and thus its repercussion in the domain of conservation biology.

1.7 Aknowledgements

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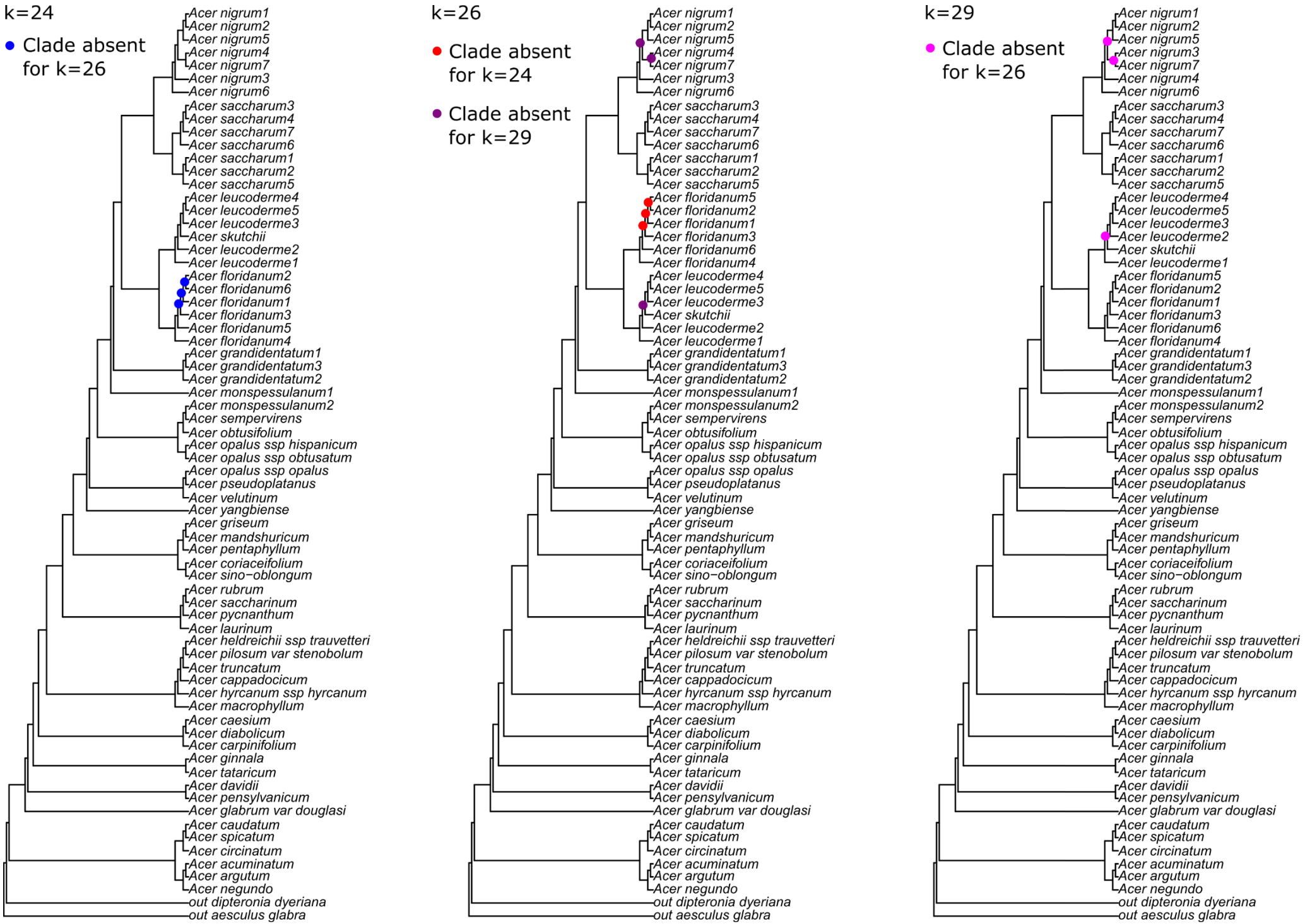

Supplementary Figure 1.4: Phylogenetic relationships obtained for k=24, k=26 and k=29.

CHAPITRE 2 :

Genomics for species delineation and conservation status determination: the Black Maple as a case study

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2.1 Abstract

Accurate taxonomic delimitation is essential for reliable conservation assessments, yet taxonomic uncertainty can undermine conservation strategies. The black maple (*Acer nigrum*) illustrates this issue, as its conservation status varies across jurisdictions and its taxonomic distinctiveness from sugar maple (*A. saccharum*) remains debated. To assess genetic differentiation between these two taxa, we analyzed high-throughput sequencing data from two sympatric stands located 700 m apart within a contiguous forest, complemented by three geographically distant, non-sympatric populations. Individuals were sampled based on diagnostic leaf morphology traditionally used to distinguish the two forms. Our results revealed clear genetic differentiation between sugar maple and black maple, supporting the distinctiveness of the black maple gene pool across both sympatric and allopatric contexts. Moreover, black maple populations in Quebec exhibited stronger genetic structure and higher levels of inbreeding than sugar maple populations at comparable latitudes. These findings support the recognition of black maple as a distinct taxonomic entity and provide the first characterization of its genetic diversity at the northern limit of its distribution. The elevated inbreeding observed in Quebec populations raises conservation concerns and highlights the need to identify the processes underlying this pattern.

Keywords: conservation genomics, GBS, black maple, sugar maple, species delineation

2.2 Introduction

2.2.1 Species delimitation and its implications for conservation

The species concept has always generated an epistemological debate in modern biology (Sites and Marshall 2003; De Queiroz 2007). The taxonomical definition of species is based on multiple, and sometimes inconsistent, species concepts relying on observations of organisms' ecology, morphology, reproduction, and genetics (Harrison and Larson 2014; Stanton et al. 2019). Ultimately, species' boundaries have been proven to be porous and unstable, as exemplified by

genomic introgression between taxonomically recognized species (Fu et al., 2022; Lazic et al., 2021) and the discovery of cryptic species (Lukhtanov 2019; Finch, Jones, and Cronn 2022). Despite these various issues with their delineation, species have a pivotal role in conservation biology. Indeed, in the urgency of managing the ongoing biodiversity crisis, there is a need for defining conservation units, and species are recognized as one of the three pillars of biodiversity by international conservation policies (Convention on Biological Diversity 2007; Coates, Byrne, and Moritz 2018). Moreover, there is a rising interest in defining intraspecific levels of conservation since intraspecific variation is threatened by local extinctions, abundance declines, and anthropogenic selection (Des Roches et al., 2021). In this context, taxonomic uncertainty around species delineation could have direct consequences on some conservation decisions, spanning both species and intraspecific levels (Morrison et al., 2009). Such decisions can have significant consequences. On the positive side, they may lead to outcomes like the recognition of several species in the genus *Sorbus*, which has sparked numerous local conservation initiatives (Meyer et al., 2005), or the genomic clarification of morphological oversplitting in hairpin banksias, allowing for a more efficient allocation of conservation resources (Wilson et al., 2022). Unfortunately, they can also result in negative outcomes, such as the extinction of distinct evolutionary units that are not recognized as species and remain unprotected at the intraspecific level (*e.g.*, snails; Hershler & Liu, 2004).

2.2.2 Taxonomic uncertainty and conservation status of black maple

An illustrative instance of taxonomic uncertainty with potentially significant consequences for conservation efforts relates to the black maple (*Acer nigrum*, Micheaux), a tree species distributed in temperate forests in Northeastern America. According to the IUCN Red List, it has been evaluated as a species of 'least concern', and it is classified as 'apparently secure' in the official species lists of the governments of Canada and the USA (IUCN Red List of Threatened Species, 2017). However, NatureServe registers black maple as either critically imperiled (S1), imperiled (S2), or vulnerable (S3) in nine of the 29 American states or Canadian provinces where it naturally occurs (NatureServe, 2024). It is threatened by habitat destruction and climatic changes, and like its closest sympatric relative, the sugar maple (*Acer saccharum*, Marshall), it will

probably be affected by the Asian longhorned beetle (*Anoplophora glabripennis*) (Natural Heritage & Endangered Species Program, 2012). Within Canada, black maple is confined to Ontario and Quebec provinces, where it is recognized as an unambiguous species. It is considered secure in Ontario but is vulnerable in Quebec where only a few populations are present at the northern limit of the species' range (Figure 2.2: Distribution map of sampling locations for testing species hypotheses. Core distribution data for each species was obtained from USDA services. Sampling design in Fasset for testing species hypotheses at a fine scale is represented in detail in Figure 2.4.Figure 2.2). Moreover, most of its populations in Quebec are located in urban areas around Montreal and are facing an increasing risk of extirpation due to habitat loss.

2.2.3 Morphological evidence and early hypotheses of hybridization

Historically, *A. nigrum* and *A. saccharum* have been described and identified as distinct species based on leaf morphological characters (Michaux, Marshall) (Table 2.1). These diagnostic traits were later used by Dansereau and Desmarais (1947) to discuss potential introgression between the two taxa. Specifically, they documented individuals exhibiting intermediate combinations of leaf morphological traits, falling between the two extreme phenotypes attributed to each species (Figure 2.1).

Table 2.1: Diagnostic morphological criteria for the identification of sugar maple and black maple

Morphological criteria of leaves	**Black maple (*Acer nigrum*)**	**Sugar maple (*Acer saccharum*)**
Blade outline	Three main lobes	Five main lobes
Blade posture	Drooping	Upright
Blade color	Dark green	Light green
Blade texture	Soft	Waxy
Blade pubescence	Dense pubescence on the abaxial surface	Glabrous; slight pubescence at the petiole–blade junction
Petiole pubescence	Dense	Glabrous
Stipules	Present	Absent

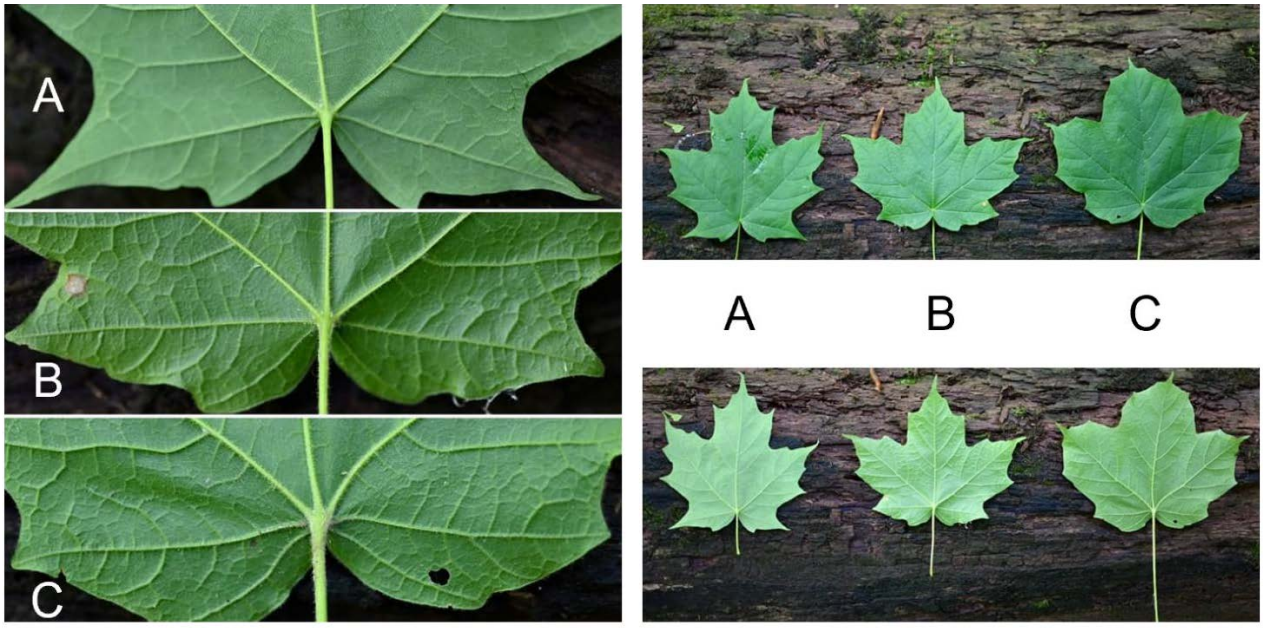

Figure 2.1 : Photos by Iain Walker. Morphological characters allowing distinction of sugar maple (A) and black maple (C): hair density of limb and petiole (right) and blade outline (left). Individuals presenting intermediates phenotypes regarding these morphologic characters (B) are considered putative hybrids by some authors but could also represent morphological variation within well delimited species.

On the basis of these observations, Dansereau and Desmarais (1947) proposed that extensive hybridization between *A. nigrum* and *A. saccharum* occurs in the northeastern portion of their range, potentially facilitated by local climatic conditions. They further suggested that because these putative hybrids generally resemble *A. saccharum*, introgression in this region could result in the erosion of genetic diversity unique to *A. nigrum* (Gabriel, 1990). However, genetic introgression was never directly quantified, and intermediate phenotypes may alternatively reflect intraspecific morphological variation. Consequently, clarifying the taxonomic boundaries between black maple and sugar maple remains essential, particularly for assessing the conservation status of black maple in Québec and informing effective management strategies.

2.2.4 Conflicting genetic and ecological evidence for species delimitation

From a genetic perspective, early studies by Skepner and Krane (1997; 1998) reported no detectable genetic differentiation between black maple and sugar maple, supporting the hypothesis that they constitute a single species. These studies, based respectively on chloroplast

DNA (cpDNA) and Random Amplified Polymorphic DNA (RAPD) markers, failed to identify genetic distinctiveness between the two taxa. However, cpDNA is highly conserved across plants, making it often unsuitable for species delineation (Petit and Excoffier 2009). Although RAPD markers can be informative when rigorously applied, the small number of polymorphic loci and individuals analyzed in these studies was insufficient to robustly test species boundaries (Nybom, 2004). Subsequent phylogenetic analyses have generally treated black maple as a subspecies of sugar maple (Gao et al. 2020; Areces-Berazain, Hinsinger, and Strijk 2021), a classification also adopted in a widely cited taxonomic revision of the genus *Acer* (van Gelderen et al., 1994). More recently, Jackson et al. (2021) used microsatellites markers to investigate population structure within the series *Saccharodendron*, which includes *A. saccharum* and five related lineages, among them *A. nigrum*. Their results revealed distinct genetic clusters corresponding to *A. nigrum* and *A. saccharum*, thereby contradicting the conclusions of Skepner and Krane (1997, 1998). Nevertheless, this study was based on three geographically distant populations (600–1500 km apart), comprising one *A. nigrum* population in Minnesota and two *A. saccharum* populations in Michigan and New York. As a result, the observed genetic differentiation may have been influenced by geographic distance rather than taxonomic divergence.

Ecologically, black maple is commonly associated with calcareous soils (Gabriel, 1990). Moreover, based on habitat descriptions and physiological responses to drought, Graves (1994) proposed that black maple represents an ecotype of sugar maple adapted to drier environments. In contrast, Hauer et al. (2021) found that black maple seedlings were more susceptible to water stress than those of sugar maple, calling this hypothesis into question. Overall, the literature suggests that black maple and sugar maple conform to the criteria of distinct species based on morphological and ecological traits (Gabriel, 1990; Godman et al., 1990; Graves, 1994; Hauer et al., 2021). However, their genetic differentiation remains unresolved, with conflicting results reported across studies (Jackson et al., 2021; Skepner & Krane, 1997, 1998).

2.2.5 Objectives, hypotheses, and spatial framework of the study

The present study aims to clarify the genetic distinctiveness of black maple (*Acer nigrum*) and sugar maple (*A. saccharum*), hereafter referred to as morphospecies, in order to inform their

taxonomic delimitation and conservation assessment. Rather than quantifying the extent of hybridization or introgression between the two taxa, our primary objective is to determine whether the two morphologically defined entities are associated with distinct genetic pools, both in sympatry and across a broader geographic context.

We investigate genetic differentiation between black maple and sugar maple using a Genotyping-by-Sequencing (GBS) approach (Elshire et al. 2011). We test two competing hypotheses regarding their taxonomic status: (i) *A. nigrum* and *A. saccharum* represent ecotypes within a single species (Graves, 1994, Skepner and Krane, 1997, 1998; Hauer et al. 2021), or (ii) they constitute two distinct species with separate gene pools (Jackson, 2021). Under the first hypothesis, we expect minimal genetic differentiation between morphospecies in sympatry, reflected by comparable F_{ST} values within and between morphospecies, the absence of discrete clustering in principal component analysis (PCA), and largely admixed or overlapping individual assignments in discriminant analysis of principal components (DAPC), as well as shared ancestry components in STRUCTURE analyses. Conversely, under the second hypothesis, genetic differentiation should mirror morphological distinctiveness, resulting in higher F_{ST} values between morphospecies than within them, the identification of two well-separated clusters in PCA, clear discrimination between morphospecies in DAPC with limited admixture, and distinct ancestry components in STRUCTURE analyses.

Genetic differentiation was evaluated at two spatial scales: a fine scale (< 1.2 km), where both morphospecies occur in sympatry within the same forest stand, and a broader regional scale incorporating individuals separated by up to 450 km. If black maple and sugar maple represent ecotypes of a single species, geographic distance is expected to exert a stronger influence on genetic differentiation than morphospecies identity. In this case, higher F_{ST} values would be observed between distant populations regardless of morphospecies, and genetic clustering would primarily reflect geographic origin. In contrast, if the two taxa represent distinct species, morphospecies membership should outweigh geographic distance, leading to consistently higher F_{ST} values between morphospecies and genetic clusters grouping individuals by morphospecies across spatial scales.

2.3 Material and method

2.3.1 Sampling design

2.3.1.1 Fine-scale sampling in sympatry

To test the species hypotheses at a fine spatial scale, sampling was conducted within a continuous temperate forest located in Fassett, Quebec, Canada, where black maple and sugar maple co-occur in sympatry. This forest is the focus of an ongoing conservation initiative aimed at establishing the Black Maple Eco-Reserve and is overseen by the Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs (MELCCFP). Within this framework, the Direction des espèces floristiques menacées has identified this forest as a high-quality black maple population based on demographic surveys conducted by accredited botanists, including assessments of population size and age structure.

In June 2021, we sampled leaf material from individuals displaying the diagnostic morphological characteristics traditionally used to distinguish black maple and sugar maple (Table 2.1; Figure 2.1A, C). Two black maple stands located approximately 700 m apart were identified within the forest. From each stand, fresh leaves were collected from 20 adult individuals, with sampled trees separated by a minimum distance of 15 m to reduce the likelihood of sampling close relatives. Leaves were immediately stored in silica gel for desiccation. For each black maple stand, two nearby sugar maple stands located 80–120 m away were selected to ensure sampling under comparable environmental conditions. The same sampling protocol was applied to sugar maple stands. Geographic coordinates of all individuals sampled at the fine spatial scale are shown in Figure 2.4a.

2.3.1.2 Broad-scale sampling in allopatry

To complement the fine-scale sympatric sampling and assess genetic patterns at a broader geographic scale, additional individuals were included from three geographically distant locations in Quebec, Canada. Fifteen supplementary A. nigrum individuals were sampled in Deux-Montagnes in July 2022. These samples were collected by a forest research technician of the Canadian Forest Service (Natural Resources Canada, Government of Canada) in response to the

imminent logging of a black maple stand, allowing the preservation of genetic material prior to site loss. In addition, eight supplementary A. saccharum individuals were included from two remote locations: Fjord-du-Saguenay (n = 4) and Sainte-Anne-du-Lac (n = 4), sampled in June and July 2018. These sugar maple samples originated from an independent sampling effort conducted by our laboratory as part of an ongoing project. These geographically distant sites were selected to provide an allopatric context for testing species hypotheses, allowing us to assess whether genetic differentiation between morphospecies persists across large spatial scales. All sampling locations, including the fine-scale design in Fassett and the three remote sites, are shown in Figure 2.2.

2.3.2 DNA extraction, library preparation, and sequencing

Total genomic DNA was extracted from approximately 50 mg of silica-dried leaf tissue using the DNeasy Plant Mini Kit (QIAGEN, Germantown, MD, USA), following the manufacturer's protocol. DNA quality and concentration were assessed prior to library preparation. Genotyping-by-sequencing (GBS) libraries were prepared following the protocol of Poland et al. (2012) at the Plateforme d'Analyses Génomiques of the Institut de Biologie Intégrative et des Systèmes (IBIS, Université Laval, QC, Canada). Briefly, genomic DNA was digested using the restriction enzymes PstI and MspI, and unique barcode adapters were ligated to each individual sample to allow multiplexed sequencing. Libraries were pooled after ligation. with the following exception: a blue Pippin (SAGE sciences) was used to size libraries before PCR amplification (elution set between 50 and 65 min, on a 2% agarose gel). An additional size-selection step was performed prior to PCR amplification using a BluePippin system (Sage Science, Beverly, MA, USA), with fragments selected between 50 and 65 min on a 2% agarose gel cassette. This step was implemented to improve library consistency across samples. PCR amplification was then conducted following the original protocol. Dual plate-level and individual-level barcoding was used to enable sequencing of pooled libraries on a shared Illumina NovaSeq S4 lane, as described in Colston-Nepali et al. (2019). Sequencing was performed at the Génome Québec Innovation Centre (Montreal, Quebec, Canada), generating paired-end reads. To estimate genotyping error rates, GBS libraries from ten randomly selected individuals were independently prepared and sequenced twice. Genotype

concordance between technical replicates was subsequently used to inform SNP filtering decisions (see Section Bioinformatics analyses).

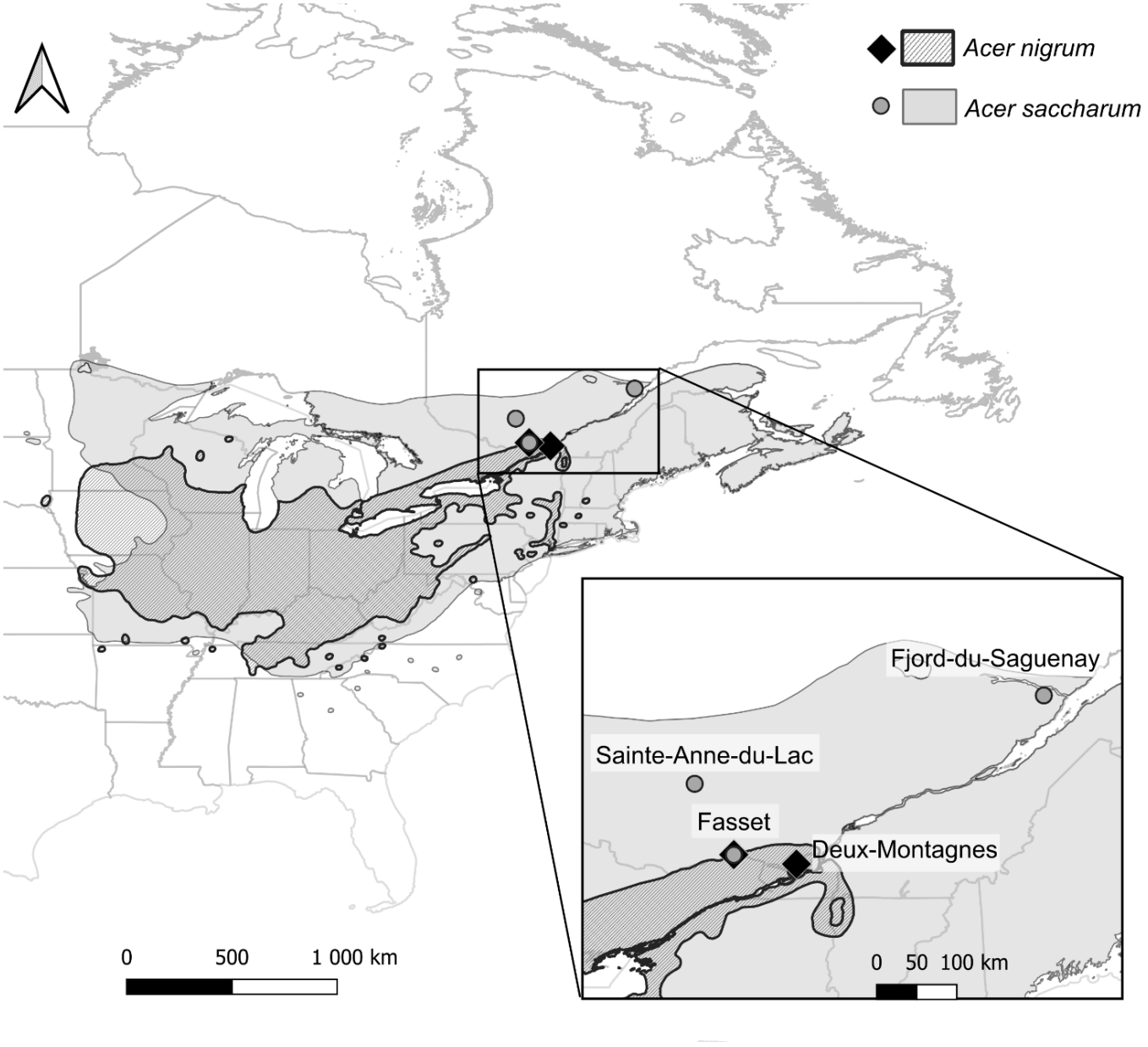

Figure 2.2: Distribution map of sampling locations for testing species hypotheses. Core distribution data for each species was obtained from USDA services. Sampling design in Fasset for testing species hypotheses at a fine scale is represented in detail in Figure 2.4.

2.3.3 Bioinformatics analyses

All scripts used for bioinformatic analysis are available in a GitHub repository.

2.3.3.1 Reads processing, alignment and SNP calling

We used Sabre (Najoshi, 2011) for demultiplexing the raw reads and removing barcodes. Then, reads were preprocessed using the fastp tool enabling overrepresented sequence analysis and base correction in overlapped regions (Chen et al., 2018). Reads shorter than 20 bp were discarded, and a mean quality threshold of 30 was applied for base calling (Chen et al., 2018).. After trimming adaptors, reads were aligned on the A. saccharum reference genome (McEvoy et al. 2022), with BWA MEM 0.7.17 (Li & Durbin, 2009). Alignments were sorted and statistics of alignment were outputted using samtools_1.17 (Li et al., 2009). Loci building and variant calling were performed in the population module in STACKS 2.62 (Rochette, Rivera-Colón, and Catchen 2019). Loci building was executed with default parameters whereas variant calling was undertaken with application of following parameters: (i) retain loci present in at least five sampling sites, (ii) retain loci present in at least 85 % of individuals in each sampling site, (iii) retain only one randomly chosen SNP per locus.

2.3.3.2 SNP filtering and linkage disequilibrium pruning

To facilitate SNPs filtering decisions for minor allele frequency (MAF), we calculated genotyping error rate for pairs of replicates on SNPs called for four different MAF value (MAF=0.001; 0.005; 0.01; 0.05) in VCFtools 0.1.16 (Danecek et al., 2011). Ultimately, retained SNPs were filtered in VCFtools 0.1.16 using the following parameters: (i) a minimum mean depth of 20X, (ii) a maximum mean depth of 500X and (iii) a minor allele frequency of 0.001 (Linck and Battey 2019). SNPs and individuals with more than 5% of missing data were excluded. After filtering, SNPs were screened in plink 1.9 (Chang et al., 2015) along windows of 50 bp every 10 bp and pruned according to a pairwise correlation threshold (r2) of 0.1 to remove loci in linkage disequilibrium. After pruning, replicated individuals with the higher proportion of missing data were removed from the dataset.

2.3.3.3 Genetic diversity and summary statistics

Individual levels of expected and observed homozygosity were computed in VCFtools 0.1.16 generating calculations of individuals expected heterozygosity (He), observed heterozygosity (Ho) and inbreeding coefficient (F) following equation described in Mooney et al. (2018). Nucleotide diversity (π) per site was computed for each morphospecies in VCFtools 0.1.16. Significance of differences of mean per individual observed and expected heterozygosity, inbreeding coefficient and per site nucleotide diversity among morphospecies was tested by t-tests in R_4.3.1.

2.3.3.4 Population structure and genetic clustering analyses

Genetic clustering was first investigated by Principal Component Analyses (PCA) on the allelic frequencies matrix with missing values replaced by the mean allele frequency in ade4_1.7-22 in R_4.3.1 (Dray and Dufour 2007). Then genetic structure was further investigated using Discriminant Analysis of Principal Components (DAPC), implemented in the R package adegenet_2.1.11 (Jombart & Ahmed, 2011), to summarize multilocus SNP variation and to maximize separation among genetic groups defined a priori according to sampling sites. Finally, in STRUCTURE_2.3.4 (Pritchard, Stephens, and Donnelly 2000; Falush, Stephens, and Pritchard 2003; 2007; Hubisz et al. 2009), exploration of the number of clusters (K) was undertaken using 10 runs for each K value from 1 to 9, with the following settings: admixture model, correlated allele frequencies, burn-in length of 10,000 and MCMC repetitions of 100,000 (mainparams file available in GitHub repository). The optimum value of K to describe genetic variation present in the data was evaluated using Evanno's protocol and Structure manual recommendations (Evanno, Regnaut, and Goudet 2005). Calculations for applying Evanno's method were undertaken in Structure Harvester (0.6.93) (Earl and vonHoldt 2012). Average individual genetic composition across STRUCTURE runs was calculated with pophelper_2.3.1 in R_4.3.1 (Francis, 2017).

2.3.3.5 Genetic differentiation analyses

Pairwise fixation indices (FST; Reynolds, Weir, et Cockerham 1983) between sampling sites were calculated using the 'diveRsity' R package (Keenan et al., 2013), with significance tested after 1000 permutations of individuals. Significance of the correlation of FST matrix with a binary matrix of

appurtenance to the morphospecies (1 = different morphospecies, 0 = same morphospecies) was tested by a Mantel test using Spearman's correlation on 999 permutations in vegan_2.6-4 in R_4.3.1 (Oksanen et al., 2022).

2.4 Results

2.4.1 Sequencing output and SNP dataset

Prior to DNA extraction and bioinformatics, we sampled 120 individuals at the fine scale and 27 remote individuals. Losses caused by laboratory and bioinformatic procedures, and the removal of replicated individuals left 40 *A. nigrum* and 79 *A. saccharum* (N=119) at the fine scale in Fasset, as well as 14 *A. nigrum* (in Deux-Montagnes) and 8 *A. saccharum* (in Fjord-du-Saguenay and Sainte-Anne-du-Lac) remote individuals (N=22). Considering both fine and broad geographic scales (N=141), we recovered on average 1.35 x 10^6 raw reads per individual (s.e. = 5.22 x 10^5) (**Erreur ! Source du renvoi introuvable.**). On average, genotyping error rates per pair of replicates were lowest for MAF=0.001 (**Erreur ! Source du renvoi introuvable.**). Bioinformatic procedures recovered 1,881 filtered and pruned SNPs located on 21 of the 388 scaffolds of *A. saccharum* identified by McEvoy et al. (2021). Mean number of SNPs per scaffold is 89.57 (min=1; max=202) and mean depth per scaffold is 62.51X (s.e. = 13.46X) (**Erreur ! Source du renvoi introuvable.**). On average, we recovered 1,871.2 variant sites per individual (s.e. = 8.8) with a mean per-individual depth of 60.8X (s.e. = 23.1X) (**Erreur ! Source du renvoi introuvable.**).

2.4.2 Genetic diversity differs between morphospecies

Differences in mean genetic diversity metrics between morphospecies were assessed using t-tests. Differences in mean expected heterozygosity ($\overline{H_E}$) among morphospecies is not significant (p-value = 0.16) while mean observed heterozygosity ($\overline{H_O}$) is significantly lower in *A. nigrum* (p-value < 0.001, **Erreur ! Source du renvoi introuvable.**, Figure 2.3). As for individual inbreeding

coefficient (F), we found that mean F is significantly higher in *A. nigrum* (p-value < 0.001, **Erreur ! Source du renvoi introuvable.**2, Figure 2.3). Regarding mean per site nucleotide diversity, it was found significantly lower in *A. nigrum* (p-value < 0.001, **Erreur ! Source du renvoi introuvable.**2).

Table 2.2: Genetic statistics for each sampling site except for nucleotide diversity (π) calculated per morphospecies only. Statistics significantly different between morphospecies are specified by an asterisk (*).

	Ho *	He	F *	π *
Black maple (*A.nigrum*)	**0.086**	**0.109**	**0.229**	**0.081**
Deux-Montagnes	0.086	0.111	0.246	
Fasset-1	0.086	0.109	0.227	
Fasset-4	0.087	0.109	0.214	
Sugar maple (*A. saccharum*)	**0.110**	**0.110**	**0.007**	**0.105**
Sainte-Anne-du-Lac	0.110	0.109	-0.005	
Fjord-du-Saguenay	0.112	0.112	0.003	
Fasset-2	0.107	0.110	0.026	
Fasset-3	0.111	0.110	-0.010	
Fasset-5	0.110	0.110	0.001	
Fasset-6	0.109	0.112	0.030	

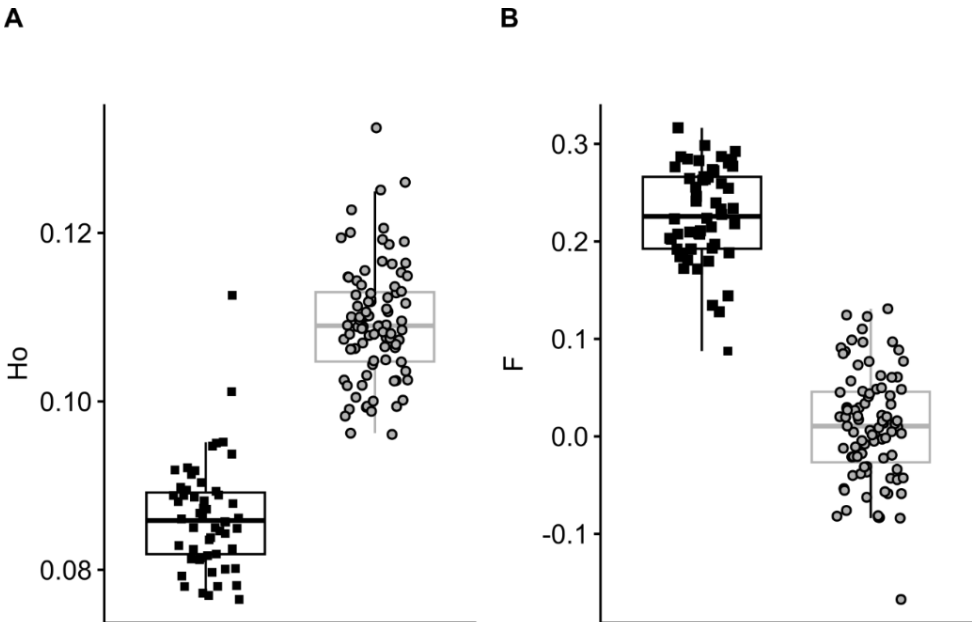

Figure 2.3 : Boxplots of individuals' observed heterozygosity (A) and inbreeding coefficient (B) for each morphospecies.

2.4.3 Fine-scale genetic structure in sympatry

Multivariate analyses were used to explore genetic structure without (PCA) and with (DAPC) a priori group assignment. PCA revealed a clear separation between *A. nigrum* and *A. saccharum* at the fine spatial scale, with PC1 explaining 10.2% of the total genetic variance and discriminating morphospecies, while PC2 captured within–*A. nigrum* spatial structure (Figure 2.4). DAPC analyses confirmed a clear genetic discrimination between *A. saccharum* and *A. nigrum* and strong population differentiation in *A. nigrum* contrasted by more admixed patterns in *A. saccharum* (Figure 2.5). Bayesian clustering analyses implemented in STRUCTURE were used to infer ancestry proportions and evaluate support for discrete genetic clusters. Evanno's calculations on ten STRUCTURE runs for 1<K<9 found higher ΔK, as well as higher likelihood with the lowest variance for K=2 (Supplementary Table 6). Moreover, individual admixture levels between morphospecies are congruent with K=2. Indeed, in the Fasset site, the mean contribution of the black cluster is 0.93 for *A. nigrum* and 0.03 for *A. saccharum* whereas the mean contribution of the grey cluster is 0.07 for *A. nigrum* and 0.97 for *A. saccharum* (Figure 2.4).

Genetic differentiation was quantified using pairwise F_{ST} values. Calculation of F_{ST} showed that genetic differentiation ($\overline{F_{ST}}$= 0.121) is higher between than within morphospecies (Table 2.3). It is noteworthy that *A. nigrum* genetic differentiation is approximately ten times higher than mean genetic differentiation within *A. saccharum* (Table 2.3; *A. nigrum* $\overline{F_{ST}}$ = 0.0438; *A. saccharum* $\overline{F_{ST}}$ = 0.0048).

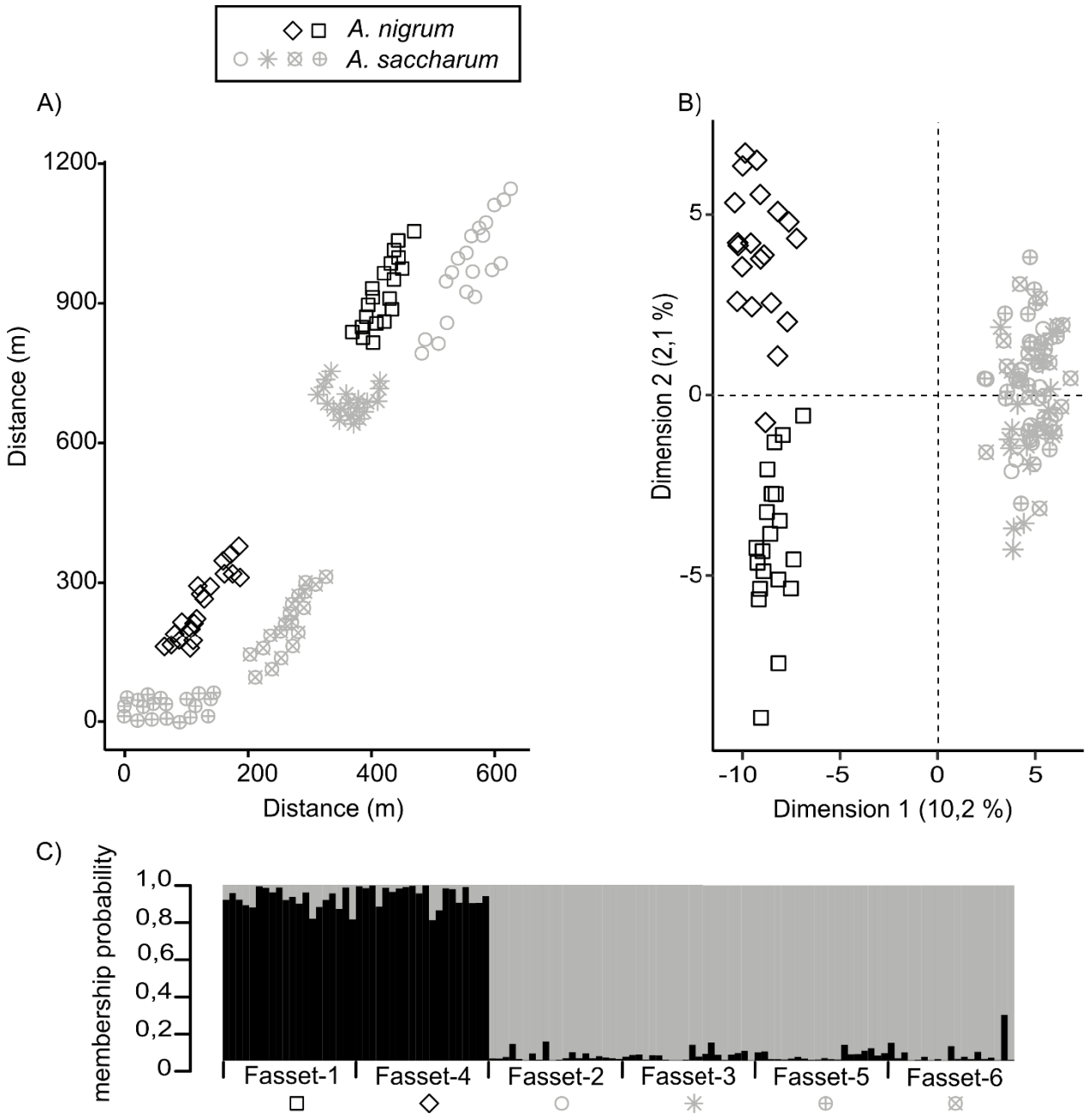

Figure 2.4 : Results for individuals of *A. nigrum* and *A. saccharum* sampled in sympatry in Fasset (Quebec, CA) : a) Sampling design for testing species hypothesis within 1.2 km, b) Principal components analysis on 1881 SNPs , c) Admixture levels as calculated by STRUCTURE at K=2.

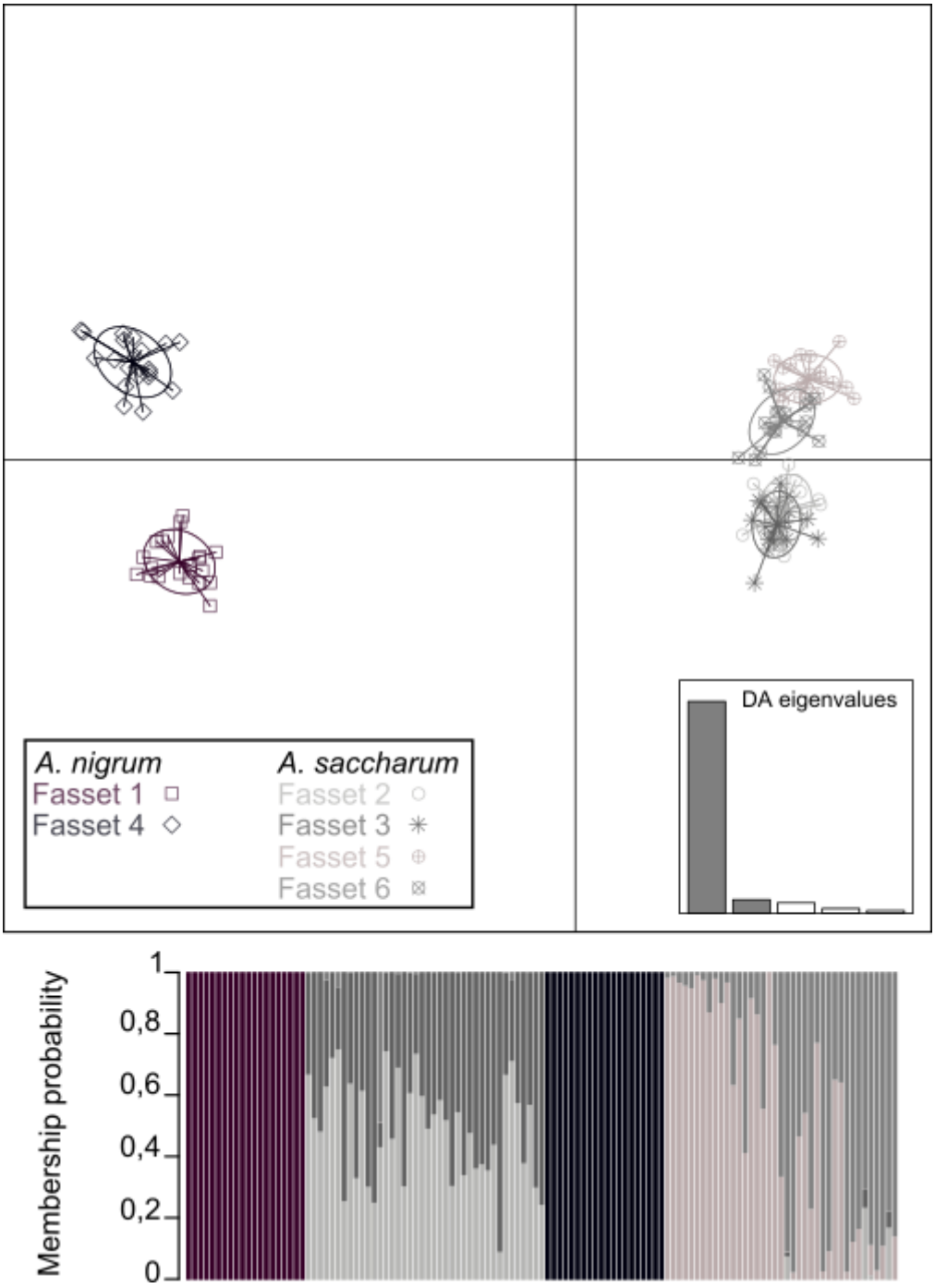

Figure 2.5 : Discriminant Analysis of Principal Components (DAPC) performed at a fine geographic scale. Discriminant axes were inferred using genetic groups defined *a priori* based on the sampling groups.

Table 2.3: Pairwise F_{ST} values (lower matrix) and geographic distance in km (upper matrix) for sampling sites of *A. nigrum* and *A. saccharum*. F_{ST} values in bold represent genetic differentiation between morphospecies. F_{ST} values in italics are not significantly different.

	A. nigrum		*A. saccharum*			
	Fasset-1	Fasset-4	Fasset-2	Fasset-3	Fasset-5	Fasset-6
Fasset-1		0.7	0.2	0.2	0.9	0.7
Fasset-4	0.0438		0.8	0.5	0.2	0.1
Fasset-2	**0.114**	**0.1269**		0.3	1.1	0.8
Fasset-3	**0.1129**	**0.1259**	*0.0021*		0.7	0.5
Fasset-5	**0.1154**	**0.1241**	*0.0049*	*0.0072*		0.3
Fasset-6	**0.1192**	**0.1298**	*0.0051*	*0.0063*	*0.0032*	

2.4.4 Broad-scale genetic structure in allopatry

At the broader geographic scale, PCA results and admixture patterns for K = 2 closely mirror those observed at the fine geographic scale (Figure 2.6). Genetic differentiation at the provincial scale follows the same overall pattern (Supplementary Table 2.7). The relative influence of morphospecies identity versus geographic distance was assessed using Mantel tests. Differentiation is significantly greater between than within morphospecies (Mantel test: r = 0.866, p = 0.0121; Supplementary Figure 2.6; Supplementary Table 2.8) and is markedly higher within *A. nigrum* ($\overline{F_{ST}}$ = 0.0864) than within *A. saccharum* ($\overline{F_{ST}}$= 0.0297). Consistent with these results, DAPC reveals contrasting within-species structure: *A. saccharum* individuals from geographically distant sampling sites exhibit admixed membership across six genetic clusters, whereas *A. nigrum*

individuals from Deux-Montagnes display pronounced genetic distinctiveness relative to those from Fassett, which show only limited admixture among themselves (Figure 2.7).

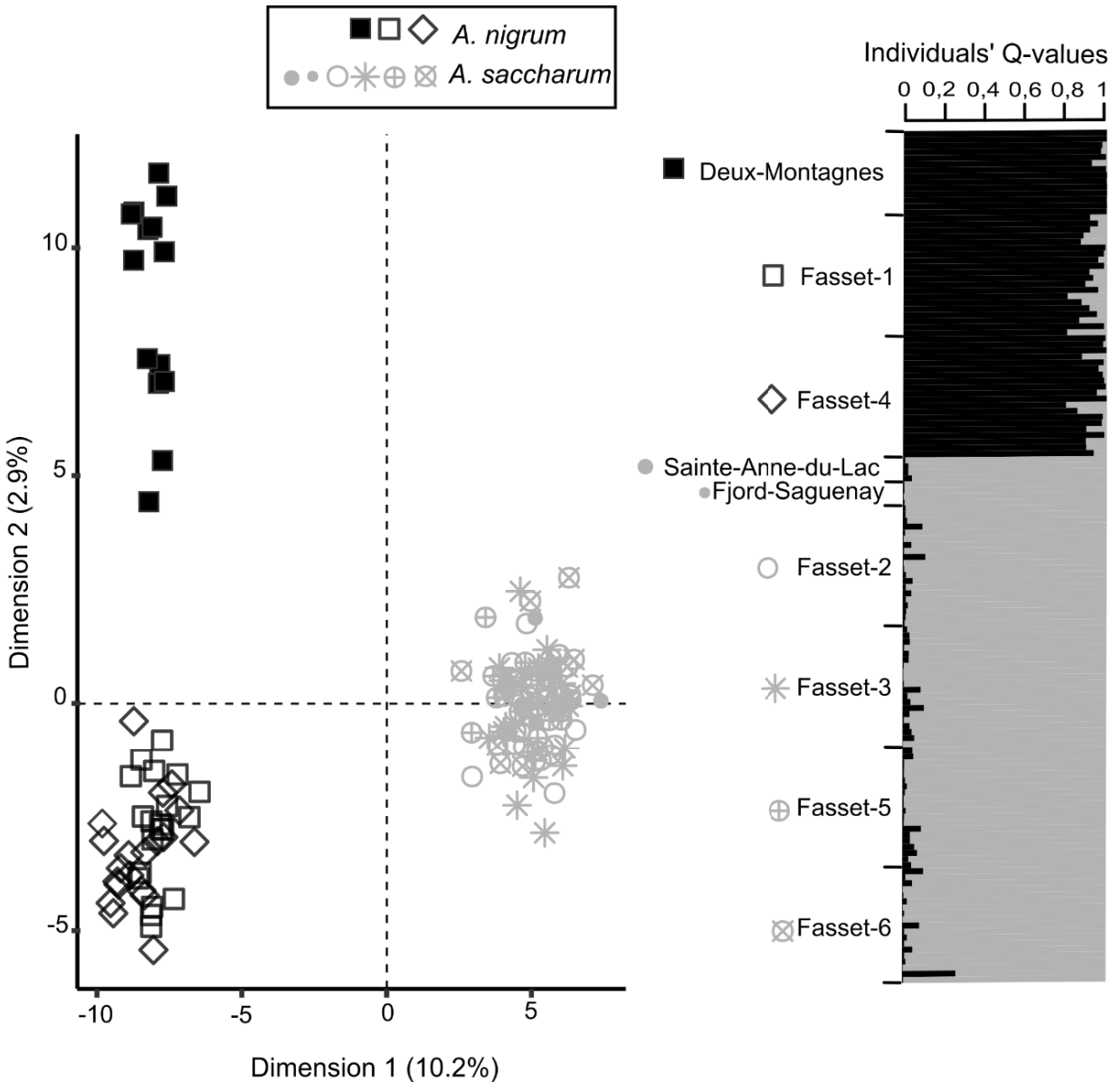

Figure 2.6 : PCA on 1881 SNPs and admixture K=2 for all individuals. Mean black cluster: 0.95 for *A. nigrum* and 0.03 for *A. saccharum*; mean grey cluster: 0.035 for *A. nigrum* and 0.97 for *A. saccharum*.

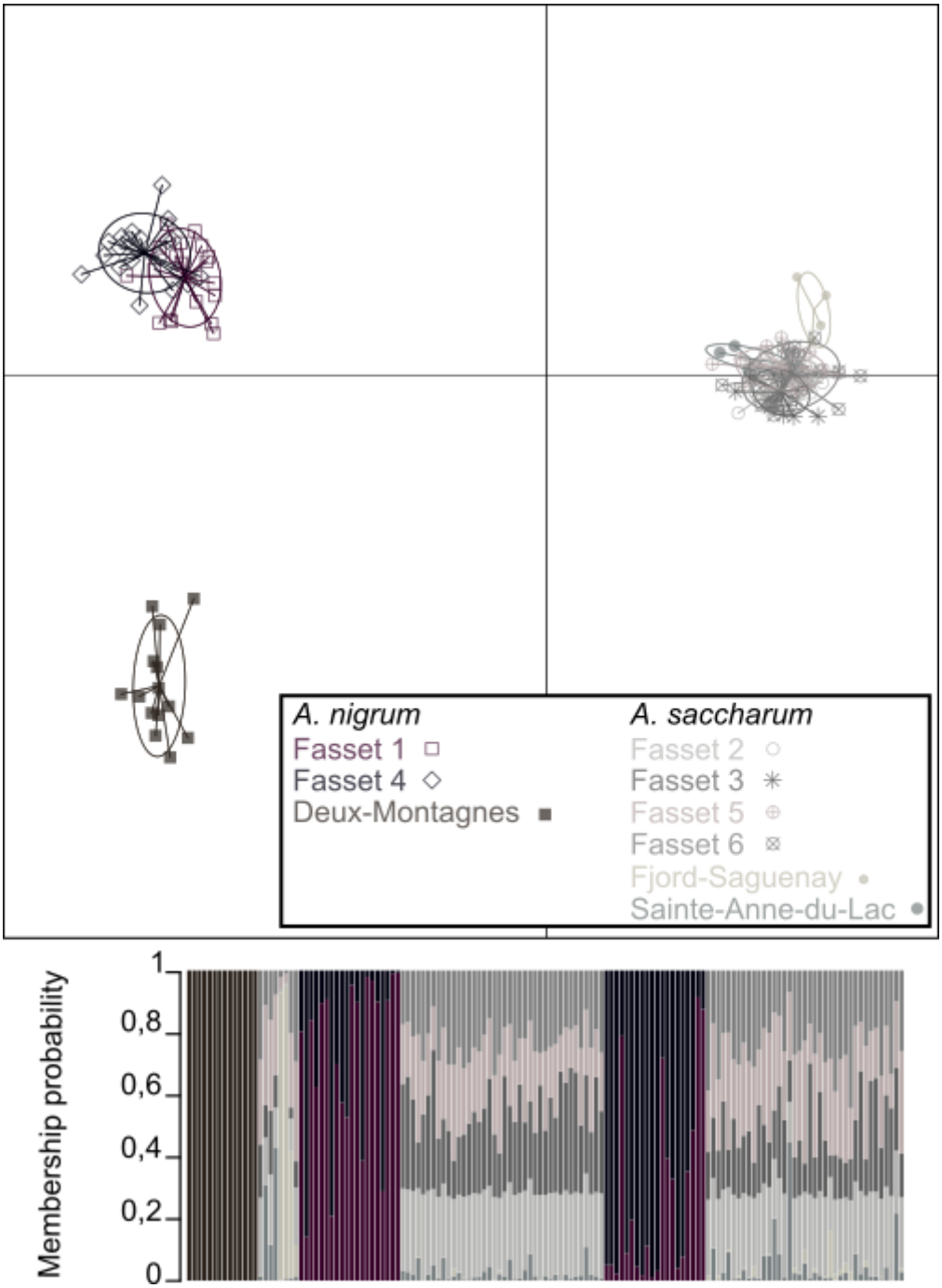

Figure 2.7: Discriminant Analysis of Principal Components (DAPC) performed at the broad geographic scale. Discriminant axes were inferred using genetic groups defined *a priori* based on sampling groups.

2.5 Discussion

2.5.1 Two sympatric species

We investigated genetic differentiation among and within two putative species of the hard maple group, *Acer saccharum* and *Acer nigrum* using a set of 1,881 SNPs generated by GBS to clarify their taxonomic designation. Our results clearly demonstrate that the morphological differences between these taxa are corroborated by distinct gene pools. Firstly, the Structure analysis suggests the existence of two distinct genetic clusters with very little admixture among them (

Figure 2.4, Figure 2.6). In the scientific literature, hybrid organisms are frequently categorized based on Q-values derived from STRUCTURE analysis, with threshold values typically ranging from 0.7 to 0.99 (van Wyk et al., 2017). In our study, individual Q-values are almost always above 0.8, with just one sugar maple individual (Fasset-6 sampling site) containing 26% of black maple ancestry (

Figure 2.4, Figure 2.6). In the literature, an introgression threshold of 0.2 (*i.e.* 80% native ancestry and 20% introgressed ancestry) has been used to support the genetic cohesion of eastern wolves (*Canis lycaon*) within the hybrid zone with grey wolves (*C. lupus*) and coyotes (*C. latrans*) (Rutledge et al., 2010). In the plant world, similar levels of introgression (ranging from 0.05 to 0.2) have been observed in taxa taxonomically classified as species, such as butternuts and oaks (Hipp et al., 2019; Hoban et al., 2009). Secondly, the PCA and DAPC results are completely congruent with the Structure analysis, and here again, two clearly distinct groups can be observed (

Figure 2.4, Figure 2.6). Lastly, pairwise F_{ST} values are significantly higher between morphospecies ($\overline{F_{ST}}$ = 0.121) than within ($\overline{F_{ST}}$ = 0.0243), as evidenced by the Mantel test (r = 0.866; p-value = 0.066) (Table 2, supplementary table S6, Supplementary Figure 2.8). These observations remain consistent when analysing both the sampling sites only at the fine scale (*i.e.* few hundred meters), or when including more distant sampling sites (up to 450 km). Ultimately, our results support the second hypothesis of species, *i.e.* black and sugar maples present enough genetic differentiation

to be considered as unique species in regards of the genetic species concept. Furthermore, our results at the fine scale (

Figure 2.4, **Erreur ! Source du renvoi introuvable.**) show that morphospecies maintain genetic cohesion even at a very restricted geographical scale where gene flow between two easily hybridizing taxa encounters no obstacles apart from partial reproductive isolation. Consequently, black maple and sugar maple appear to qualify as distinct species under the cohesion species concept, which aligns with the biological species concept but includes genetic and/or demographic exchangeability. It is important to note, however, that our results do not rule out hybridization between the two species since we identify some admixed individual. We propose that the typical phenotypes at either end of the morphology spectrum, representing black and sugar maples (**Erreur ! Source du renvoi introuvable.**), correspond to distinct and cohesive genetic groups with characteristic ecological traits (Hauer et al., 2021). Considering the application of four species concepts, we argue that black and sugar maples should be recognized as separate species rather than ecotypes. Moreover, their sympatry across most of their range precludes their classification as subspecies (Grant, 1981; Mayr, 1999). Finally, while we recognize that hybridization might occur between these species, a phenomenon commonly observed among distinct tree species (*e.g.*, red and silver maples: Saeki et al. 2011, oaks: Li et al. 2021, and willows: Fogelqvist et al. 2015), it seems not extensive enough to compromise their genetic integrity. Ultimately, our study brings evidence that the two species can coexist in sympatry while remaining predominantly pure. It is beyond the scope of our study to quantify introgression between them, and identify the potential isolating mechanisms, and a more exhaustive sampling of the two species range will be needed in the future to clarify these issues.

2.5.2 Differences in genetic structure

Based on our results, sampling sites of *A. nigrum* are more differentiated than those of *A. saccharum*. Indeed, using the same set of SNPs, *A. nigrum* sampling sites less than one kilometer distant present higher pairwise F_{ST} values than *A. saccharum* ones separated by ~130 km (**Erreur !**

Source du renvoi introuvable.; **Erreur ! Source du renvoi introuvable.**). The PCA analysis at the fine scale confirms this result since the two *A. nigrum* sampling sites are clearly separated on the second axis, with just one individual of Fasset-4 clustering with Fasset-1 (

Figure 2.4). Similarly fine-scale DAPC indicates pronounced genetic structuring among black maple sampling sites in Fassett, whereas sugar maple individuals display largely admixed assignment probabilities across the four sampling sites (Figure 2.5). This overall pattern of stronger genetic structuring within black maple is further supported by both PCA and DAPC analyses at the broad geographic scale (Figures 2.6 and 2.7). In the PCA, sugar maple individuals sampled across large geographic distances (up to 450 km apart) cluster together, whereas black maple individuals sampled over more limited distances (130 km apart) segregate into two distinct genetic clusters (Figure 2.6). Consistent with these results, DAPC reveals contrasting within-species patterns: sugar maple individuals, spanning distances from 700 m to 450 km, exhibit highly admixed membership across six shared genetic clusters, while black maple sampling sites correspond to three genetic clusters. One of these clusters is unique to the Deux-Montagnes site, whereas the two others are shared and show partial admixture among black maple individuals from Fassett (Figure 2.7). In addition to this higher genetic differentiation, black maples are also significantly more inbred than sugar maples (Figure 2.3, **Erreur ! Source du renvoi introuvable.**2). Furthermore, for the same set of SNPs, *A. nigrum* display lower per site nucleotide diversity and lower observed heterozygosity compared to *A. saccharum* (**Erreur ! Source du renvoi introuvable.**2). Weak connectivity among groups of individuals can cause higher inbreeding coefficients as well as lower observed heterozygosity (Sork and Smouse 2006). However, these two genetic metrics are only slightly correlated (Slate et al., 2004), as the former expresses the tendency of individuals to reproduce with kins based on their multilocus genotypes while the latter measures differences in local alleles frequences. Overall, genetic structuring and inbreeding levels observed in *A. nigrum* is rather unexpected in long-lived perennials, outcrossing and wind-pollinated seed plants (Kling & Ackerly, 2021; Lowe et al., 2015; Nybom, 2004), suggesting an impediment to gene flow among *A. nigrum* sampling sites even at a fine scale, that has no effect in *A. saccharum*. However, the origin of this contrasted genetic structure between these two closely related maple species needs further study.

Indeed, both species are wind-pollinated (Gabriel 1990; Godman, Yawnay, and Tubbs 1990) implying long-distance gene flow with homogenizing effects on genetic variation, as evidenced multiple times for *A. saccharum* (Perry et Knowles 1989; Geburek 1993; Gunter et al. 2000; Khodwekar et al. 2015; Graignic, Tremblay, and Bergeron 2018). Moreover, their flowering season, from April to May, overlap (*A. nigrum* starts a few weeks earlier; Gabriel 1990). Furthermore, both species are monoecious, with male and female flowers on the same plant (Gabriel 1990; Godman, Yawnay, and Tubbs 1990). Given the similitudes in flower and pollen morphology, as well as timing and vector for pollen dispersion, it is unlikely that phenological differences produce the observed higher genetic differentiation and inbreeding in *A. nigrum*. However, the much higher abundance of *A. saccharum* in Quebec suggests that its pollen is present in greater quantities in pollen clouds. Such difference in pollen relative abundance could increase pollen competition and imped reproduction of *A. nigrum* through pollen swamping as observed in a Andean cedar species (Martyniuk, Morales, and Aizen 2015).

Another possible mechanism contributing to the dissimilar genetic structuring between sampling sites of *A. saccharum* and *A. nigrum* is seed dissemination. Seeds of *A. saccharum* are known to be wind-dispersed around a mean distance of 100 meters (Godman, Yawnay, and Tubbs 1990). On the contrary, seeds of *A. nigrum* generally fall close to the parent tree (Ministry of Natural Ressources, 2000), travelling long distances only in high winds (Gabriel, 1990) or by water (Ministry of Natural Ressources, 2000). As evidenced in the European ash *Fraxinus excelsior,* this weaker dispersal capacity can lead to high genetic differentiation between populations and inbreeding levels within populations in tree species (Heuertz et al., 2003).

Moreover, in Quebec, *A. nigrum* has been subjected to substantial anthropogenic pressures in recent decades, particularly through logging, as the species naturally occurs in regions experiencing high real estate development (e.g., the Montreal area). Such anthropogenic disturbances may have caused demographic contractions in black maple populations, potentially resulting in genetic bottlenecks. Similar demographic events in other tree species have been associated with increased population divergence, reduced genetic diversity, and, in some cases, elevated levels of inbreeding (*Fagus sylvatica* : Jump and Peñuelas 2006, *Taxus baccata* : Dubreuil

et al. 2010 and *Juglans cinerea* : Hoban et al., 2014). Accordingly, the patterns of genetic diversity observed in *A. nigrum* populations in Quebec may reflect, at least in part, the species' vulnerable status and its recent demographic history in the province.

Finally, populations of *A. nigrum* in Quebec are at the northern limit of the species range. Several studies support the notion that populations at the northern edge of species distribution ranges are subject to distinct evolutionary forces, especially drift and environmental selection (Chhatre & Rajora, 2014; Lesica & Allendorf, 1995; Tollefsrud et al., 2016). For black maple, it is likely that environmental factors influence a selection mechanism at the seedling level, since a recent study demonstrated that the establishment of seedlings of *A. nigrum* is limited by substrate moisture levels (Hauer et al. 2021). We conducted botanical surveys at the Fasset sampling sites to examine variations in botanical composition between sites dominated by sugar maple and those dominated by black maple (Supplementary Figure 2.9). Our results supported the previous findings, as companion species of *A. nigrum* were typical cues of a humid temperate forest habitat. Establishment of black maple at northern latitudes depends on stochastic events influenced by wind direction as well as the nature of the substrate where the seeds land, which may consequently have created genetic bottlenecks. Ultimately, genetic bottlenecks caused by colonization combined with strong evolutionary forces of the species' northern edge could also lead to the pattern of high divergence, inbreeding and low genetic diversity observed in *A. nigrum* as it has been observed for other wind-pollinated tree species (*Fagus crenata*, Kitamura et al. 2015; *Quercus rubra*, Götz, Rajora, and Gailing 2022).

2.6 Conclusion and future research

In this study, we demonstrate that two North American maples, sugar maple (*A. saccharum*) and black maple (*A. nigrum*), maintain clearly distinct gene pools even when occurring in sympatry. These results provide strong genetic support for the recognition of black maple as a distinct species and indicate the presence of effective barriers to gene flow operating across two geographic scales. The persistence of such genetic differentiation in sympatric contexts suggests

that reproductive isolation between these morphospecies is maintained despite opportunities for interspecific contact. Although our analyses did not explicitly quantify hybridization or introgression, they highlight the need to further investigate the mechanisms underlying the maintenance of species boundaries between *A. saccharum* and *A. nigrum*. Integrative approaches combining robust morphometric analyses with genomic data will be essential to accurately assess the frequency and extent of hybridization and introgression. In addition, several pre- and postzygotic barriers potentially contributing to reproductive isolation, such as pollen–pistil compatibility or hybrid seed viability, remain to be explored to provide a more comprehensive understanding of reproductive isolation in this species complex. Beyond reproductive barriers, ecological differentiation may also contribute to the observed genetic structure. While black maple has been described as ecologically distinct in the literature, its ecological niche remains poorly characterized. The strong genetic differentiation observed between black and sugar maple populations occupying seemingly similar habitats raises the possibility that fine-scale environmental heterogeneity or microhabitat variation influences their spatial distribution and limits gene flow. Investigating ecological niche differentiation at very fine spatial scales would therefore be a valuable avenue to test the ecological species hypothesis in this system. From a conservation perspective, our results reveal high levels of genetic differentiation and inbreeding within *A. nigrum* populations in Quebec, consistent with its designation as a vulnerable species in the province. These patterns may reflect the combined effects of restricted gene flow, demographic history, and the species' position at the northern edge of its distribution. Further studies are needed to disentangle the relative contributions of interspecific pollen competition, habitat restriction, limited seed dispersal, historical exploitation, and range-edge dynamics to the observed genetic structure. Assessing genetic connectivity and inbreeding across the broader range of *A. nigrum* will be critical to determine whether Quebec populations are genetically isolated or part of a larger metapopulation. Finally, given the broad zone of sympatry between sugar maple and black maple across North America, future work should aim to delineate the geographic extent of potential hybridization zones and to compare patterns of genetic diversity in allopatric and sympatric populations. By providing a comprehensive portrait of genetic variation across the ranges of both species, such studies will contribute to a deeper understanding

of species boundaries in long-lived forest trees and will support more informed conservation and management strategies for black maple.

2.7 Aknowledgements

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Supplementary Table 2.4 : Sampling site's metadata comprising geographical coordinates, mean number of raw reads, mean number of variant sites and mean depth per individual after filtering and pruning procedures.

Location	N	Morpho-species	Mean Latitude	Mean Longitude	Mean raw reads (SE)	Mean number filtered variant sites (SE)	Mean depth/individual after filtering (SE)
Deux-Montagnes	14	*A. nigrum*	45.532	-73.892	814344.7 (305052.65)	1869.6 (18.20)	43 (16.22)
Sainte-Anne-du-Lac	4	*A. saccharum*	46.770	-75.461	1689281.8 (341961.83)	1872.0 (0)	83.7 (16.19)
Fjord-Saguenay	4	*A. saccharum*	48.133	-70.078	1799034.8 (186242.3)	1867.0 (0)	76.4 (6.97)
Fasset 1	20	*A. nigrum*	45.682	-74.854	1733174.8 (592006.01)	1874.3 (2.9)	75.4 (25.96)
Fasset 2	20	*A. saccharum*	45.683	-74.852	1293482.1 (367227.69)	1871.2 (4.31)	54.2 (15.48)
Fasset 3	20	*A. saccharum*	45.680	-74.855	1280096.0 (498962.94)	1871.4 (3.31)	54.3 (22.04)
Fasset 4	20	*A. nigrum*	45.676	-74.858	1552508.9 (501550.37)	1873.3 (4.05)	70.1 (23.07)
Fasset 5	20	*A. saccharum*	45.675	-74.859	1319328.5 (409530.47)	1870.9 (9.85)	61.6 (18.21)
Fasset 6	19	*A. saccharum*	45.676	-74.856	1156609.3 (527695.01)	1867.9 (12.83)	53.9 (25.05)

Supplementary Table 2.5 : Genotyping error rates calculated on SNPs dataset before filtering for depth and missingness. The raw dataset is filtered out for four different MAF values. The two replicates of the individual (ID) are subset of the SNPs datasets and genotype for each position is extracted. Genotypes for each position are compared among pairs. Position with divergent call among pairs are counted and their number is divided by the total number of genotypes available for analyses.

ID	MAF=0.001 N genotypes = 7,191	MAF=0.005 N genotypes = 5,848	MAF=0.01 N genotypes = 4,435	MAF=0.05 N genotypes = 2,191
QCPAPI01.01	0.0160	0.0171	0.0183	0.0233
QCPAPI01.02	0.0090	0.0087	0.0092	0.0100
QCPAPI01.03	0.0145	0.0150	0.0156	0.0128
QCPAPI01.04	0.0757	0.0891	0.1080	0.1716
QCPAPI01.05	0.0104	0.0106	0.0117	0.0105
QCPAPI04.20	0.0259	0.0299	0.0309	0.0351
QCPAPI05.01	0.0256	0.0272	0.0298	0.0288
QCPAPI05.02	0.0236	0.0246	0.0253	0.0274
QCPAPI05.03	0.0731	0.0817	0.0961	0.1328
QCPAPI05.04	0.0179	0.0190	0.0201	0.0237
MEAN	**0.0292**	**0.0323**	**0.0365**	**0.0476**

Supplementary Table 2.6 : Coverage statistics of variant positions found along scaffolds identified by McEvoy et al. (2022). Name and length describe the scaffolds.

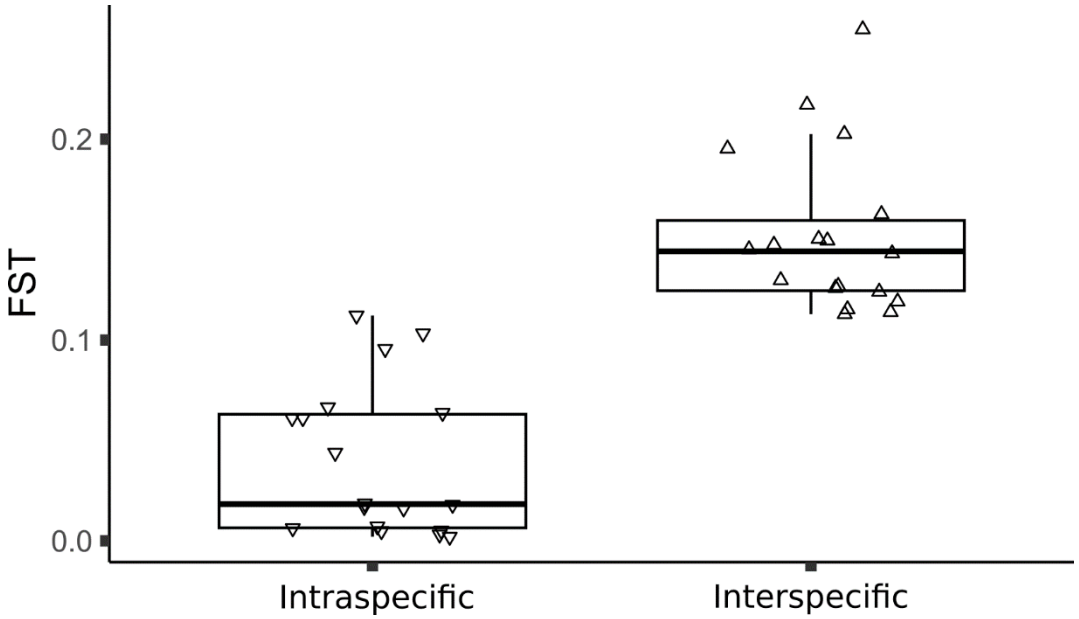
Name	Length (bp)	Number of SNPs	Mean Depth	SE
acsa_001	50470275	133	59.75	18.72
acsa_002	55331383	192	59.50	22.01
acsa_003	65226167	155	61.74	23.63
acsa_004	68459104	186	62.33	20.79
acsa_028	985231	1	73.79	0.00
acsa_082	175453	1	36.17	0.00
acsa_172	36426	1	66.01	0.00
acsa_222	86876	1	107.24	0.00
acsa_367	50000	1	67.82	0.00
acsa_369	250000	1	43.11	0.00
acsa_370	250000	2	66.16	11.66
acsa_379	50000	1	62.60	0.00
acsa_380	33992772	105	60.50	20.99
acsa_381	41337728	144	59.99	21.01
acsa_382	37324271	112	61.80	20.53
acsa_383	40376603	135	63.84	20.92
acsa_384	46969240	202	61.90	20.82
acsa_385	36639500	102	59.32	22.13
acsa_386	43483981	136	58.70	19.70
acsa_387	42652568	135	63.23	20.26
acsa_388	45724731	135	57.17	19.48
TOTAL	**609872309**	**1,881**		
MEAN	**29041538.52**	**89.57**	**62.51**	**13.46**

Supplementary Table 2.7 : Evanno's calculations

# K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	-96,234.34	2.683	NA	NA	NA
2	10	-90,813.15	10.6521	5,421.19	5,503.12	516.623041
3	10	-90,895.08	294.0525	-81.93	2,939.56	9.996718
4	10	-93,916.57	7,183.5224	-3,021.49	22,599.13	3.145968
5	10	-119,537.19	26,416.8797	-25,620.62	1,854.34	0.070195
6	10	-147,012.15	31,802.9911	-27,474.96	53,213.01	1.673208
7	10	-121,274.1	35,734.52	25,738.05	22,634.84	0.633417
8	10	-118,170.89	44,208.2398	3,103.21	13,095.08	0.296214
9	10	-101,972.6	4,797.8153	16,198.29	NA	NA

Supplementary Table 2.8 : Pairwise F_{ST} values (lower matrix) and geographic distance in km (upper matrix) for sampling sites of *A. nigrum* and *A. saccharum*. F_{ST} values in bold represent genetic differentiation among morpho-species. F_{ST} values in italics are not significantly different from 0 (**Erreur ! Source du renvoi introuvable.**). Values in the dashed line delimited area correspond to the fine scale in Fasset, Qc. Mean F_{ST} between morphospecies = 0.1520. Mean F_{ST} within *A. nigrum* = 0.0864. Mean F_{ST} within *A. saccharum* = 0.0297.

	A. nigrum			*A. saccharum*					
	Deux-Montagnes	Fasset-1	Fasset-4	Fasset-2	Fasset-3	Fasset-5	Fasset-6	Sainte-Anne-du-Lac	Fjord-Saguenay
Deux-Montagnes		76.9	77	76.7	76.9	77	76.9	183.3	410.1
Fasset-1	0.1033		0.7	0.2	0.2	0.9	0.7	129.6	454.4
Fasset-4	0.1122	0.0438		0.8	0.5	0.2	0.1	130.1	455
Fasset-2	**0.1504**	**0.114**	**0.1269**		0.3	1.1	0.8	129.6	454.2
Fasset-3	**0.1474**	**0.1129**	**0.1259**	*0.0021*		0.7	0.5	129.8	454.6
Fasset-5	**0.1452**	**0.1154**	**0.1241**	*0.0049*	*0.0072*		0.3	130.3	455.2
Fasset-6	**0.1496**	**0.1192**	**0.1298**	*0.0051*	*0.0063*	*0.0032*		130.2	454.9
Sainte-Anne-du-Lac	**0.2026**	**0.1431**	**0.1626**	*0.0186*	*0.018*	*0.0162*	*0.0169*		433.2
Fjord-Saguenay	**0.2547**	**0.1953**	**0.2172**	0.0612	0.0612	0.0612	0.0664	0.0955	

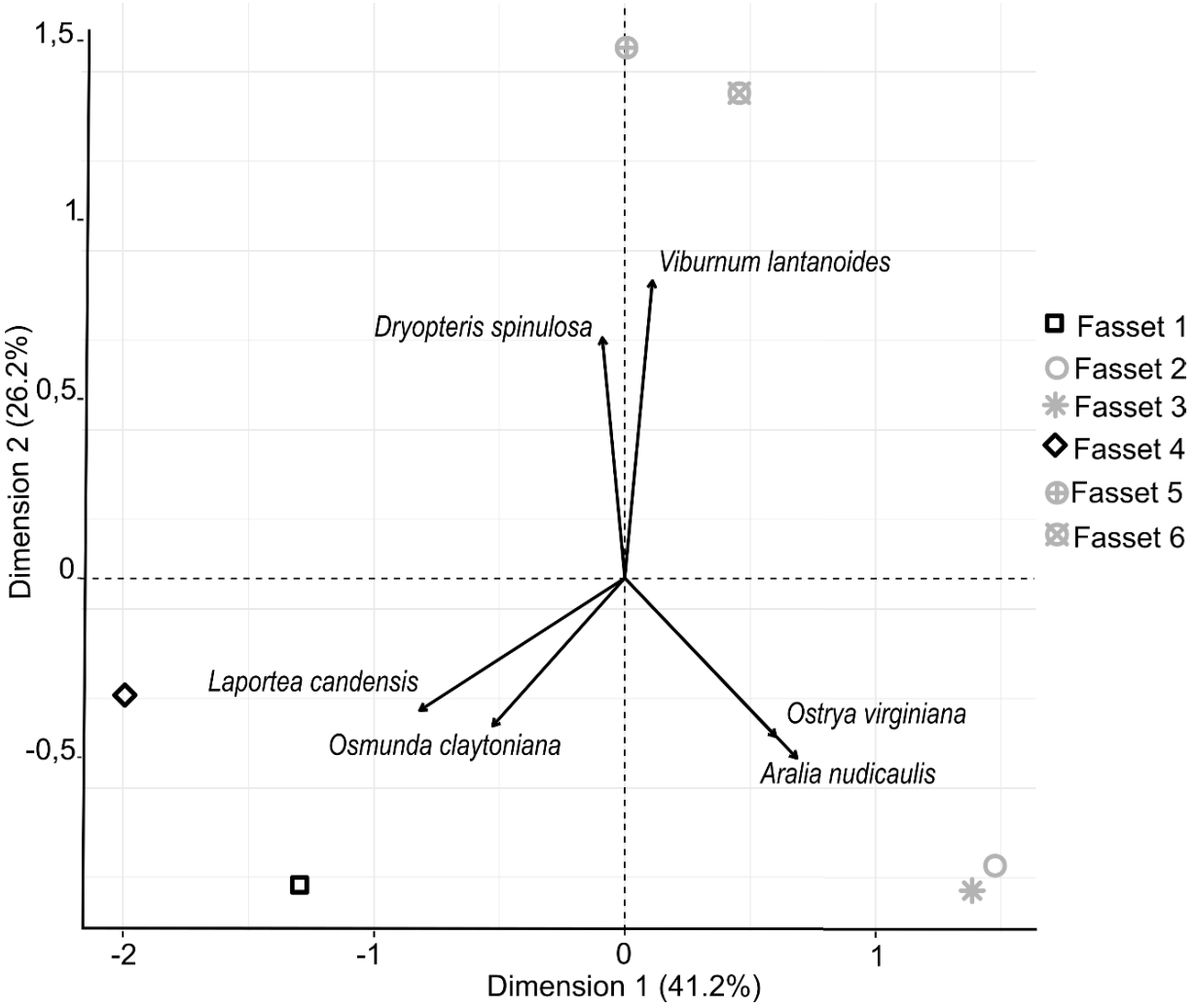


Supplementary Figure 2.8 : Pairwise intraspecific and interspecific F_{ST} values.

Supplementary Table 2.9 : Pairwise F_{ST} values with bias corrected (BC) confidence interval (CI) calculated by diveRsity_1.9.90 (R 4.2.2). Confidence interval with lower limit < 0 imply unsignificant F_{ST} value. In the "test" column: "AmongNig" is for pairs of *A. nigrum* sampling sites. "AmongSac" is for pairs of *A. saccharum* sampling sites and "Between" is for pairs of sampling sites including both morphospecies.

	actual	BC_Lower_95%CI	BC_Upper_95%CI	Test
Deux-Montagnes vs. Fasset 1	0.1033	0.0823	0.1297	AmongNig
Deux-Montagnes vs. Fasset 4	0.1122	0.0905	0.1368	AmongNig
Fasset 1 vs. Fasset 4	0.0438	0.0297	0.0604	AmongNig
Sainte-Anne-du-Lac vs. Fjord-Saguenay	0.0955	-3.00E-04	0.2535	AmongSac
Sainte-Anne-du-Lac vs. Fasset 2	0.0186	-0.0293	0.0927	AmongSac
Sainte-Anne-du-Lac vs. Fasset 3	0.018	-0.0303	0.0919	AmongSac
Sainte-Anne-du-Lac vs. Fasset 5	0.0162	-0.0326	0.0896	AmongSac
Sainte-Anne-du-Lac vs. Fasset 6	0.0169	-0.0332	0.0939	AmongSac
Fjord-Saguenay vs. Fasset 2	0.0612	0.0215	0.1333	AmongSac
Fjord-Saguenay vs. Fasset 3	0.0637	0.0247	0.1347	AmongSac
Fjord-Saguenay vs. Fasset 5	0.0612	0.0199	0.1361	AmongSac
Fjord-Saguenay vs. Fasset 6	0.0664	0.0262	0.1379	AmongSac
Fasset 2 vs. Fasset 3	0.0021	-0.0091	0.0153	AmongSac
Fasset 2 vs. Fasset 5	0.0049	-0.0056	0.0188	AmongSac
Fasset 2 vs. Fasset 6	0.0051	-0.0055	0.0193	AmongSac
Fasset 3 vs. Fasset 5	0.0072	-0.0039	0.023	AmongSac
Fasset 3 vs. Fasset 6	0.0063	-0.005	0.0223	AmongSac
Fasset 5 vs. Fasset 6	0.0032	-0.0081	0.0166	AmongSac
Deux-Montagnes vs. Sainte-Anne-du-Lac	0.2026	0.1473	0.2855	Between
Deux-Montagnes vs. Fjord-Saguenay	0.2547	0.2089	0.3279	Between
Deux-Montagnes vs. Fasset 2	0.1504	0.134	0.1727	Between
Deux-Montagnes vs. Fasset 3	0.1474	0.1307	0.1692	Between
Deux-Montagnes vs. Fasset 5	0.1452	0.1279	0.164	Between
Deux-Montagnes vs. Fasset 6	0.1496	0.1325	0.1702	Between
Sainte-Anne-du-Lac vs. Fasset 1	0.1431	0.094	0.2136	Between
Sainte-Anne-du-Lac vs. Fasset 4	0.1626	0.1128	0.2357	Between
Fjord-Saguenay vs. Fasset 1	0.1953	0.156	0.258	Between
Fjord-Saguenay vs. Fasset 4	0.2172	0.1784	0.2796	Between
Fasset 1 vs. Fasset 2	0.114	0.1029	0.1279	Between
Fasset 1 vs. Fasset 3	0.1129	0.1018	0.1282	Between
Fasset 1 vs. Fasset 5	0.1154	0.1036	0.1304	Between
Fasset 1 vs. Fasset 6	0.1192	0.1056	0.1362	Between
Fasset 2 vs. Fasset 4	0.1269	0.1145	0.1433	Between
Fasset 3 vs. Fasset 4	0.1259	0.1132	0.1421	Between

Fasset 4 vs. Fasset 5	0.1241	0.1115	0.139	Between
Fasset 4 vs. Fasset 6	0.1298	0.115	0.1482	Between



Supplementary Figure 2.9 : Principal component analysis of botanical surveys undertaken in Fasset. Botanical surveys of the herbaceous and shrubby section, as well as tree seedlings, were undertaken in plots measuring 6 m by 6 m located in the approximate center of the sampling sites in Fasset. Species surveyed were classified into five categories of percentage of presence in the plot (1%, 5%, 10%, 15%, 25%) and principal component analysis was used to investigate variation in botanical composition between sites. Variation in botanical composition in sampling sites of Fasset is explained at 67.4% by the first two axis. *A. nigrum* sampling sites separated by 700 meters regroup in the same area of the two-dimensional space whereas sampling sites of *A. saccharum* regroup by stands (Fasset 2 regrouped with Fasset 3 and Fasset 5 regrouped with Fasset 6). The six botanical species with higher contribution are shown by arrows.

CHAPITRE 3:

Landscape genomics in sugar maple unravels adaptation to local environment despite high level of gene flow

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3.1 Abstract

Migratory rates of tree species are expected to lag behind the rapid changes in environmental conditions due to climate changes. Given the ecological, economic, and cultural importance of sugar maple in Quebec, we focused on the detection of genomic variation associated with climate adaptation in this species. Using a combination of outlier's detection approach and Genetic-Environment-Association (GEA), signatures of local adaptation were investigated at two geographical scales: a global one, including 23 populations distributed across Quebec province, and a local one represented by eight pairs of populations situated on an elevation gradient. We provided evidence of a homogenized distribution of nuclear genomic variation among populations of Quebec (F_{st} = 0.0226 ± 0.013), probably due to high gene flow caused by wind-pollination. However, despite this lack of genetic structure across the study area, evidence of local adaptation was detected. We identified genomic positions associated with minimum temperature, precipitation, wind speed, vegetation cover type, clay's cation exchange capacity and solar radiation across the province. Furthermore, we highlighted effect of elevation on local selection regimes. Overall, our results constitute evidence for the adaptive potential of sugar maple, allowing local adaptation despite high levels of gene flow.

Keywords : Acer saccharum, climate change, GEA analysis, genotyping by sequencing, population genomics, temperate forest

3.2 Introduction

The pace of current climate change constitutes a threat for natural ecosystems and primary production by changing local environmental conditions rapidly (Aitken, Yeaman, Holliday, Wang, & Curtis-McLane, 2008) and asymmetrically over large territories and climatic gradient (Rotbarth et al., 2023). To persist in a changing environment, organisms depend on both their ability to follow their ecological niche through migration, and their capacity to adapt to local climatic conditions (Davis and Shaw 2001; de Lafontaine et al. 2018). In this context, it is urgent to document the adaptive capacity of species, which includes their adaptive potential, their dispersal ability and their phenotypic plasticity (Beever et al., 2016). Moreover, significant concern rises as various species may lack the migration capacity to cope with future climatic changes (Berg et al., 2010; Parmesan, 2006). Species with long life span, characterized by slow growth and delayed sexual maturity, are anticipated to experience migration rates that lag behind the rapid pace of climate change (Bisbing et al., 2021). Many tree species fit these characteristics (McKenney et al., 2007) and, as selective pressure on trees populations rises (Weed, Ayres, and Hicke 2013; Millar and Stephenson 2015), climate change threatens the numerous ecological, and socio-economical services they provide globally (Aitken, Yeaman, Holliday, Wang, & Curtis-McLane, 2008; Bang et al., 2017; Ellison et al., 2005; Hamrick, 2004).

With this rising concern, literature documenting adaptive capacity of trees species has increased, bringing evidence for the persistence of trees species in a changing environment through phenological changes (Chmielewski and Rötzer 2001), moving their range to cooler regions at higher latitudes or altitudes (Thom et al. 2017; Solarik et al. 2018), phenotypic plasticity (Valladares et al., 2014) or rapid adaptation based on standing genetic variation (Ahrens et al., 2019; Barrett & Schluter, 2008; Capblancq, Fitzpatrick, et al., 2020). Especially, great attention has been brought on the characterisation of local adaptation in trees populations (Visser 2008; Hoffmann and Sgro 2011; Alberto et al. 2013; Fitzpatrick and Keller 2015; Capblancq 2023). Understanding local adaptation relies on the identification of phenological and physiological traits, as well as the underlying genetic variation, associated with persistence of organisms in local environments (Aubin et al., 2016; Benomar et al., 2016). As these traits and genetic variation have

been selected through time they constitute putative targets to selection in a new environmental context, thus documenting the adaptive potential of populations. Furthermore, this knowledge serves as input to increase efficiency of assisted migration programs, favouring the movement of pre-adapted genotypes (Aitken & Whitlock, 2013).

Tree species are often distributed across large climatic gradients that enhance divergent selection and shapes local adaptation (Leimu and Fischer 2008; Savolainen, Pyhäjärvi, and Knürr 2007; Sork et al. 2013; Capblancq et al. 2023). The most efficient approach to detect local adaptation is the establishment of reciprocal transplants or common gardens experiments (Blanquart et al., 2013b; de Villemereuil et al., 2016). However, these are not easily undertaken on tree species, since selection events occur on long timespans (up to 200 years) and that number of viable descendants, *i.e.* the measure of fitness (Kawecki & Ebert, 2004), is hardly measurable during a financially realistic research experiment. Nevertheless, current cost-effective methods for obtaining high-quality genome scale data offer another way to identify genomic variants related to adaptive differences among tree populations (Prunier et al. 2011; Gautier 2015; Ahrens, Byrne, and Rymer 2019). Two popular methods for identifying putative locally adaptive genomic variants rely on studying the distribution of genomic variation among populations, that is influenced by several evolutionary processes, including natural selection. The first one, commonly called “outliers’ approaches (OA)”, calculates the differentiation of genomic positions shared among populations located on environmental gradients and identifies variants with high differentiation as candidates to natural selection (Joost et al., 2007). The second one, usually designated as “genetic-environment association (GEA)”, is an exploratory approach that searches for correlations between populations allele frequencies and quantitative measures of their local environments (Forester et al., 2017; Hoban et al., 2016; Rellstab et al., 2015). When correctly executed, these approaches are well suited for studying local adaptation occurring in natural populations of forest tree species (Hoban et al., 2010; Pluess et al., 2016; Rajora et al., 2016).

This study investigates local adaptation within populations of an emblematic North American tree species, the sugar maple (*Acer saccharum* Marshall). Because this species is wind-pollinated (Roussy, 2014), high levels of gene flow are expected to homogenize allele frequencies across the

landscape, thereby limiting the effects of selection and potentially counteracting local adaptation (Lenormand, 2002). In wind-pollinated tree species, the constraining effect of gene flow on local adaptation is expected to be particularly strong, as selection typically acts on many loci with small individual effects rather than on a few major-effect genes (Neale & Kremer, 2011; Tigano & Friesen, 2016; Yeaman & Guillaume, 2009). However, more recent theories suggest that gene flow plays a more nuanced role in adaptation as the migration of alleles, whether adaptive or maladaptive, can influence the fitness of local populations. Thus, depending on the balance between selection and migration, gene flow may either facilitate or hinder local adaptation (Capblancq, Fitzpatrick, et al., 2020; Welch & Jiggins, 2014). Indeed, evidence for local adaptation in wind-pollinated trees has been reported from common garden experiments and landscape genomics approaches (Cox et al., 2011; Savolainen et al., 2007). In this study, we investigate whether local adaptation occurs despite high gene flow among sugar maple populations in Quebec.

Sugar maple is a dominant tree species in temperate mixed wood forests of North-East America, with a core range distribution lying between 60° to 95° of longitude West, and 35° to 50° of latitude North (Figure 3.1) (Godman et al., 1990). It presents a substantial ecological role, as an important component of six forest cover types of North-East America (Eyre, 1980) with a strong influence in nitrogen cycling (Lovett & Mitchell, 2004). Additionally, it displays a significant socio-economic importance as a valuable source for timber and non-timber products, including maple syrup and attractive fall colors (Hinrichs, 1998; Houston, 1999; Statistics Canada, 2022; Zasada & Strong, 2003). Furthermore, due to its wide distribution range, sugar maple encounters a diverse array of environmental conditions and has therefore been considered a suitable model for studying species' responses to climate change (Putnam & Reich, 2017). Finally, its genetic structure has already been investigated using multiple genetic markers and there is clear consensus across literature on high gene flow among sugar maple populations at both local and range-wide scales due to wind-pollination (Perry and Knowles 1989; Gunter et al. 2000; Graignic, Tremblay, and Bergeron 2018). Overall, sugar maple presents all the characteristics of a good candidate to investigate local adaptation patterns despite high levels of gene flows. Moreover, its

decline in parts of its range over the last four decades (Boakye et al., 2023; St Clair et al., 2008) also justifies that its adaptive potential should be further documented.

Sugar maple has a broad ecological amplitude but grows best in cool, humid climates and on well-drained loams (Godman et al., 1990; Horsley et al., 2002). Common gardens experiments, at different scales, under outdoor and indoor conditions suggest the presence of local adaptation to environment in sugar maple populations. Considering the latitudinal temperature gradient across the sugar maple's range, Solarik et al. (2016) found evidence for local adaptation of seed germination to temperature. Between 43° and 49° of latitude North (*i.e.* the northern part of sugar maple's range), bud phenology was significantly associated with the local minimum April temperature (Guo et al. 2020) and plastic across latitudes (Guo et al., 2023). Guo et al. (2020) also suggested that patterns of local adaptation to temperature could be influenced by both latitude and longitude depending on the proximity of the sea, underlining a putative effect of a longitudinal humidity gradient (Rowe 1972). Furthermore, with temperature increase, sugar maple's range is expected to expand northward and upward (Graignic et al., 2014). However, the elevational and northern range edges of sugar maple are both limited by the ecotone with the boreal forest; while sugar maple is a dominant component of the deciduous forest at low elevations and latitudes, it gives way to boreal forest dominated by spruce (*Picea spp.*) and balsam fir (*Abies balsamea* Mill.) at highest elevations and latitudes (Brown & Vellend, 2014). In Quebec province, literature supports that the northward or upward expansion of sugar maple's range will be restrained by the acidic edaphic conditions of the boreal forest and there is evidence of the restraining effects of low base cations (Carteron et al., 2020), low soil pH (Solarik et al. 2018) and low nutrient availability (Collin et al., 2017) on sugar maple's establishment at higher latitudes or altitudes. Despite the identification of climatic and edaphic variables participating in local adaptation of sugar maple, identification of genomic variants associated with these variables in natural populations using next generation sequencing (NGS) data has never been undertaken yet. Furthermore, it is unknown how levels of gene flow will interact with selective pressures of local environment in natural populations.

To assess the adaptive potential of sugar maple, we examined genetic structure and signatures of local adaptation in Quebec populations using Genotype-by-Sequencing (GBS; Elshire et al. 2011) data. With respect to genetic structure, we evaluated two alternative hypotheses. First, given that sugar maple is a wind-pollinated tree species (Roussy, 2014), populations are expected to be highly connected through extensive gene flow. Second, as reported for other North American tree species (Jaramillo-Correa et al., 2009), sugar maple populations may exhibit genetic structure shaped by historical isolation of lineages during postglacial recolonization. Under the first hypothesis, we predict high levels of admixture among individuals and low genetic differentiation among populations (Perry and Knowles 1989; Gunter et al. 2000; Graignic, Tremblay, and Bergeron 2018). In contrast, under the second hypothesis, we expect the recovery of genetic clusters differentiating populations along an east–west gradient. Moreover, while literature suggests that high levels of gene flow have a constraining effect on local adaptation (Kremer et al., 2012), it has not been empirically tested on sugar maple. We used a sampling design allowing the comparison of signals of local adaptation at two geographical scales: the province scale with populations up to 650 km apart and local scale represented by height pairs of populations situated on an elevation gradient. According to previous results on adaptation in sugar maple, we expect: i) the distribution of adaptive genetic variation at the province scale is associated with variations in temperature, humidity and soil properties (base cations, pH and nutrient availability) (Collin et al. 2018; Solarik et al. 2016; 2018; Carteron et al. 2020; Guo et al. 2020; 2023), ii) given the high level of gene flows, we expect weaker signal of adaptation at the local scale than at the province scale due to constraining effects of migration on selective divergence.

3.3 Material and methods

3.3.1 Sampling

Mature *A. saccharum*, at least 15 meters apart, were sampled in 24 locations (referred hereafter as populations) across Quebec during July and August 2018 (Figure 3.1). Among these populations, 16 were paired along an altitudinal gradient (from 191 m to 814 m; with an elevation variation

between 101 m to 364 m - **Erreur ! Source du renvoi introuvable.**), and are designated in analysis a s population pairs (N=8; **Erreur ! Source du renvoi introuvable.**) for the detection of genomic adaptation at a local scale in opposition to the provincial scale (*i.e.* all populations). Healthy-looking leaves from tips of sunlit branches were collected, immediately placed in paper envelopes, and stored in plastic containers with silica gel desiccant. Sampling effort varied between 20 to 41 mature trees per population leading to a total of 655 sampled individuals, 355 of which allowed to test local adaptation along elevation gradients.

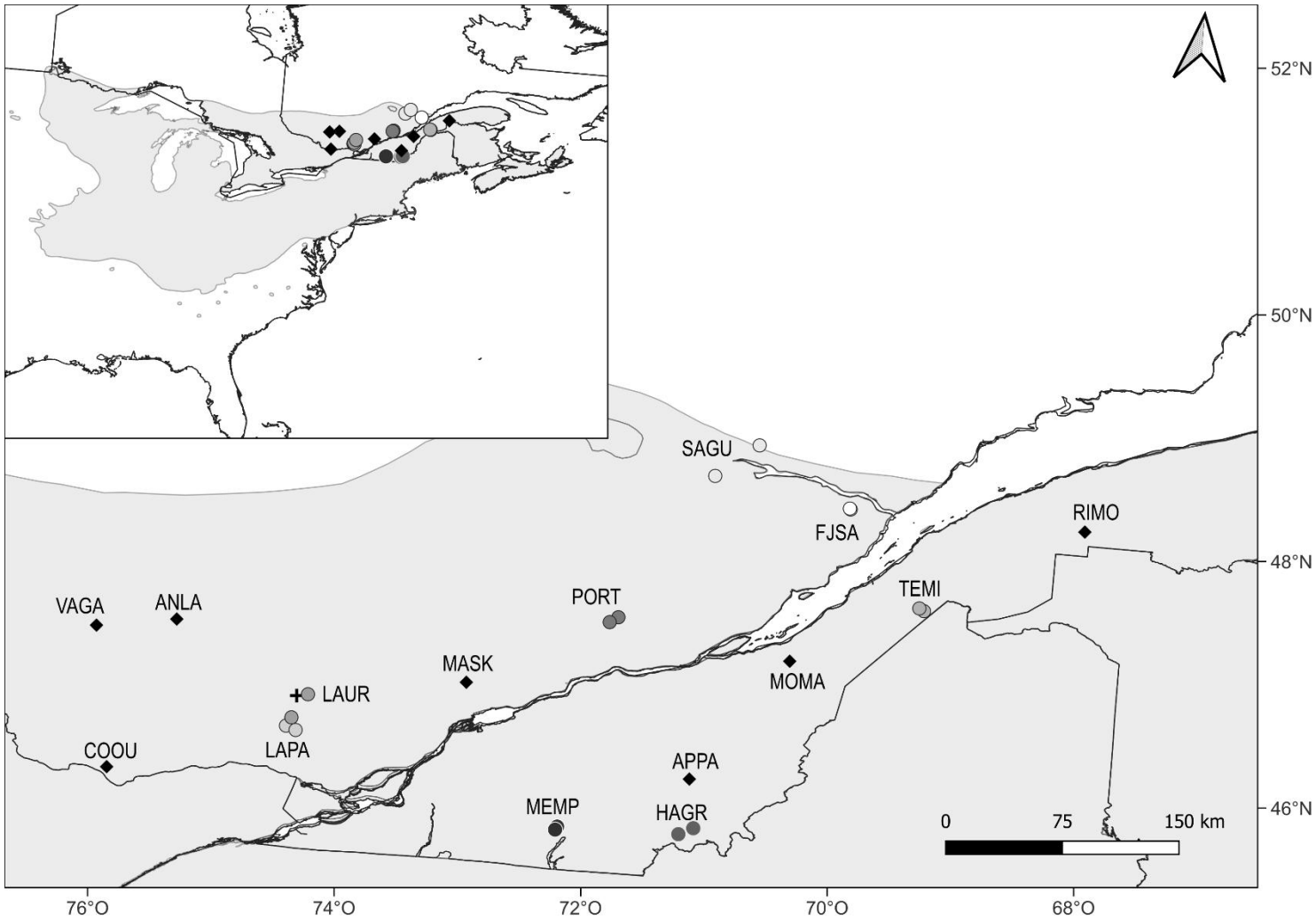

Figure 3.1: Distribution map of populations used in this study; populations in pairs for testing local adaptation at a local scale are symbolized by grey dots and stand-alone populations are symbolized by black squares. Populations and pairs of populations are labeled as listed in **Erreur ! Source du renvoi introuvable.**. The black cross represents a population removed d uring laboratory procedures. Core distribution range of *Acer saccharum* (grey shade) is as published by USDA services.

3.3.2 DNA extraction, library preparation and sequencing

Total genomic DNA was extracted from 50 mg of dried leaves according to two complementary protocols. The first one is the CTAB extraction protocol provided by Sandra Boles (Aboul-Maaty & Oraby, 2019) with the following changes: i) steps related to liquid azote were dismissed since dried, instead of frozen, leaves were grinded, ii) DNA pellets washing step was executed with 70 % ethanol for 30 min, iii) DNA pellets were resuspended in low TE buffer overnight at 4°C. The second protocol corresponds to the manufacturer's instructions of the QIAGEN Plant Mini Kit extraction (Qiagen, Germantown, MD). Libraries for Illumina GBS were prepared following Poland et al. (2012) at the Plateforme d'Analyses Génomiques of the Institut de Biologie Intégrative et des Systèmes (IBIS, Université Laval, QC, Canada) with the following exception: a blue Pippin (SAGE sciences) was used to size libraries before PCR amplification (elution set between 50 and 65 min, on a 2% agarose gel). Plate and individual barcoding was used to enable sequencing on a shared Illumina NovaSeq S4 lane as described in Colston-Nepali et al. (2019). Sequencing was performed at the Centre d'expertise et de services Génome Québec (Montreal, QC, Canada). For three randomly chosen individuals (**Erreur ! Source du renvoi introuvable.**), we prepared the GBS l ibrary twice to estimate genotyping error rates by comparing called genotypes in replicated samples and facilitating decision-making at the SNPs filtering step.

3.3.3 SNP calling

We used Sabre (Najoshi, 2011) for demultiplexing the raw reads and remove barcodes. All scripts used for bioinformatic analysis on individual's fastqs are available in a GitHub repository. Reads were preprocessed using the fastp tool enabling overrepresented sequence analysis and base correction in overlapped regions (Chen et al., 2018). Reads shorter than 20 bp were discarded, and a mean quality threshold of 30 was applied for base calling (Chen et al., 2018). After trimming adaptors, reads were aligned on the *A. saccharum* reference genome (McEvoy et al. 2022), with BWA MEM 0.7.17 (Li & Durbin, 2009). Alignments were sorted and statistics were outputted using samtools_1.17 (Li et al., 2009). Loci building and variant calling were performed by applying the

ref.map.pl script in STACKS 2.62 (Rochette et al., 2019). Loci building was executed with default parameters whereas variant calling was undertaken with application of following parameters: (i) retain loci present in at least five populations, (ii) retain loci present in at least 85 % of individuals in each population, (iii) retain one random SNP per locus. To facilitate SNPs filtering for minor allele frequency (MAF), we calculated genotyping error rate for pairs of replicates with four different MAF value (MAF=0.001; 0.005; 0.01; 0.05) in VCFtools 0.1.16 (Danecek et al., 2011). Ultimately, retained SNPs were filtered in VCFtools 0.1.16 using the following parameters: (i) a minimum mean depth of 20X, (ii) a maximum mean depth of 500X and (iii) a minor allele frequency of 0.001 (Linck & Battey, 2019). SNPs and individuals with more than 15% of missing data were excluded. After filtering, SNPs were screened in plink 1.9 (Chang et al., 2015) along windows of 50 bp every 10 bp and pruned according to a pairwise correlation threshold (r^2) of 0.1 to remove loci in linkage disequilibrium. After pruning, replicated individuals with the highest proportion of missing data were removed from the dataset. Furthermore, we cleaned variant sites for putative paralogs by removing SNPs for which more than 85% of individuals were heterozygous. Additionally, to identify putative clones in the populations, pairwise individual relatedness values were computed in VCFtools 0.1.16 following (Manichaikul et al., 2010). We identified pairs of distinct individuals with relatedness probability > 0.4 and remove one of the individuals. Observed and expected heterozygosity as well as F_{IS} statistic per populations were calculated in dartR_2.9.7 in R_4.3.1 (Gruber et al., 2018; R Core Team, 2022). We also calculated F_{IS} statistic per loci using hierfstat_0.5-11 in R_4.3.1 (Goudet & Jombart, 2004) to identify and remove loci with F_{IS} value equal to 1.

3.3.4 Genetic structure

First, model free genetic clustering was investigated with Principal Component Analysis (PCA) in ade4_1.7-22 (Dray and Dufour 2007) in R_4.3.1. To further look for genetic clustering, estimation of number of ancestral populations (K) was undertaken in Admixture_1.3.0 (Alexander and Lange 2011). Following guidelines, values of K from 1 to 23 (the total number of populations studied after bioinformatic filtering; **Erreur ! Source du renvoi introuvable.**) were tested. The value that m

inimized the prediction error was considered as the number of ancestral populations (Alexander and Lange 2011). Pairwise fixation indices (F_{ST}; Reynolds, Weir, et Cockerham 1983) between populations were calculated using the 'diveRsity' R package (Keenan et al., 2013), with significance tested after 1000 permutations of individuals. Isolation by Distance (IBD) of all populations was evaluated by a Mantel test using non-parametric Spearman correlation test and 9999 permutations in R_4.3.1 (vegan_2.6-4; Oksanen 2022) on linearized F_{ST}, i.e. $F_{ST}/(1-F_{ST})$ (Rousset, 1997) and geographic distances, calculated using Vincenty ellipsoid method (Vincenty, 1975) with R package geosphere_1.5-18 (Hijmans, Williams, and Vennes 2019).

3.3.5 Detection of local adaptation

At both geographic scales, we undertook outlier proportions analysis in pcadapt_4.3.5 (Luu, Bazin, and Blum 2017) in R 4.3.1. In essence, this analysis tests how much individual SNPs are associated with clustering, assuming extreme values of SNP coordinates are outliers and supposedly indicative of local adaptation (Privé et al., 2020). The significance of outliers SNPs is based on Mahalanobis distance and associated p-value were adjusted using FDR-method (Benjamini & Hochberg, 1995; François et al., 2016).

For the GEA approach, we constituted an environmental database describing abiotic factors of populations' environment. We downloaded monthly climate data measured from 1970 to 2000 at the spatial resolution of 30" (~ 1 km2) from the WorldClim database (https://www.worldclim.org/data/worldclim21.html, Fick and Hijmans 2017). Downloaded climatic data comprised 19 bioclimatic predictors for ecological applications (BIO1-BIO19; O'Donnell and Ignizio 2012) as well monthly means for precipitation (mm), temperature (average, maximum and minimum; °C), solar radiation ($kJ.m^{-2}.day^{-1}$), wind speed ($m.s^{-1}$), water vapor pressure (kPa). Monthly measures of climatic variables were averaged into annual means, and we also added the standard deviation of these variables in the data set. Additionally, elevation data at the same spatial resolution (~ 1 km^2) was downloaded. Furthermore, we extracted 18 variables describing soil composition of the top layer (depth 0 to 20 cm) from the Harmonized

Global Soil database v.2.0 (FAO & IIASA, 2023). Finally, we extracted vegetation cover categories and slopes from populations coordinates in the database provided by the Ministère des Ressources Naturelles et des Forêts of Québec (MRNF, 2023) and exposition of slopes were inferred in QGIS_3.30 (QGIS Development Team, 2023).

Allele counts at the populations level were computed at the provincial scale (*i.e* the complete dataset) ('adegenet') and tested for association with environmental variables by redundancy analyses (RDA) in vegan_2.6-4 in R_4.3.1 (Oksanen et al., 2022). RDA determines how groups of loci vary in response to environmental conditions, considered as explanatory variables, and thus detects processes that result in multi-locus molecular signatures (Forester et al., 2017; Rellstab et al., 2015). A procedure of selection of explanatory variables was undertaken following the stepwise method 'ordistep' implemented in vegan_2.6-4 in R_4.3.1 (Oksanen et al., 2022) where the significance of each variable was tested by 9999 permutations. To avoid putative collinearity among the selected variables, we investigated correlations among pairs of variables using psych_2.4.6.26 (Revelle, 2024) in R_4.3.1 and removed one variable out of pairs of variables showing a correlation of 0.7 or more (Ukrainetz et al., 2011). Finally, variables with variance influence factor (VIF) higher than 10 were removed from the model (Gareth, 2013). The percentage of genetic variation explained by the final ordination model was estimated via an adjusted r-squared ($_{adj}R^2$) (Legendre and Legendre 2012) and the significance of canonical axes was tested by 9999 permutation tests in vegan_2.6-4 in R_4.3.1 (Legendre et al. 2011; Oksanen et al. 2022). Specific loci associated with environmental adaptation were searched using a procedure proposed by Capblancq et al. (2018) that estimates loci's association to principal components by calculating a p-value based on a Mahalanobis distance (*i.e.*, the distance estimated between the z-scores of the locus on the K first principal component and the mean of all the loci z-scores on the same set of principal components). Finally, we controlled FDR by transforming the p-values into q-values using the procedure of the 'qvalue' R package (Storey et al., 2021). We kept the loci with q-values less than 10^{-3}, which corresponds to a false discovery rate of 0.1%.

For the local instance, Discriminant Analyses of Principal Component (DAPC) was undertaken in adegenet_2.1.10 in R_4.3.1 to investigate whether populations within a pair were genetically discriminated along the elevation gradient. For each pair of populations, we first examined the discrimination of SNPs along 10 axes in the Principal Component Analysis (PCA), followed by one discriminant function in the Discriminant Analysis (DA). To identify SNPs involved in the discrimination of populations along the elevation gradient, we extracted positions that contributed to at least 1% of the discriminant axis and considered them as candidate SNPs.

Candidate SNPs identified by all approaches were summarized in a list of unique candidates and a second RDA was undertaken on these specific loci, providing an 'adaptively enriched genetic space' (Steane et al., 2014), allowing the identification of environmental variables that are the most correlated with putative adaptive variation. Unique candidates were then compared to the reference genome (McEvoy et al., 2022). To do so, segments of 500 pb prior and after candidate SNPs were intersected with the reference genome using bedtools_2.27.1 (Quinlan & Hall, 2010) allowing to establish lists of genes intersecting with at least 10% of the 1000bp sequences containing candidates SNPs.

3.4 Results

3.4.1 Sequencing results

On average, genotyping error rates per pair of replicates were lower for MAF=0.001 (**Erreur ! S ource du renvoi introuvable.**). After the filtering steps, our dataset contained 559 individuals, distributed in 23 populations of at least 12 individuals. Among those populations, 16 represent the local scale as they are paired along elevational gradients. On average, we recovered 2.3 x 10^6 raw reads per individual (s.e. = 7.9 x 10^5). The variant calling procedure identified a total of 707 SNPs, with an average of 676 SNPs (s.e.= 22.1) per individual and a mean depth per site and per individual of 93.1X (s.e.= 32.4X). **Erreur ! Source du renvoi introuvable.** provide the sequencing s tatistics per population.

3.4.2 Summary statistics and genetic structure

Mean observed ($\overline{H_O}$) and mean expected heterozygosity ($\overline{H_E}$) are not significantly different ($\overline{H_O}$ = 0.036; $\overline{H_E}$ = 0.037; p-value = 0.1454). Mean F_{IS} statistic is significantly positive ($\overline{F_{IS}}$ = 0.055; p-value = 2.888 x 10^{-7}; **Erreur ! Source du renvoi introuvable.**). The first two dimensions of PCA e xplain 4.6 % of genomic variation; respectively 2.4% for the first axis and 2.2% for the second. Regardless of their population of origin, individuals are closely plotted in the two-dimension space created by these first two principal components (Figure 3.2). The lowest admixture cross-validation error score in Admixture is obtained for K=1 and composition plots of individuals for 2<K<10 show high levels of admixture among populations (Supplementary Figure 3.5). Mean pairwise F_{ST} at the provincial scale is 0.0226 (min = -0.0008; max = 0.0659; s.e. = 0.013; Supplementary). The IBD analysis revealed that matrix of geographic distances among populations and matrix of linearized population pairwise F_{ST} values are not significantly correlated (Mantel r statistic = 0.02732; p-value = 0.3854).

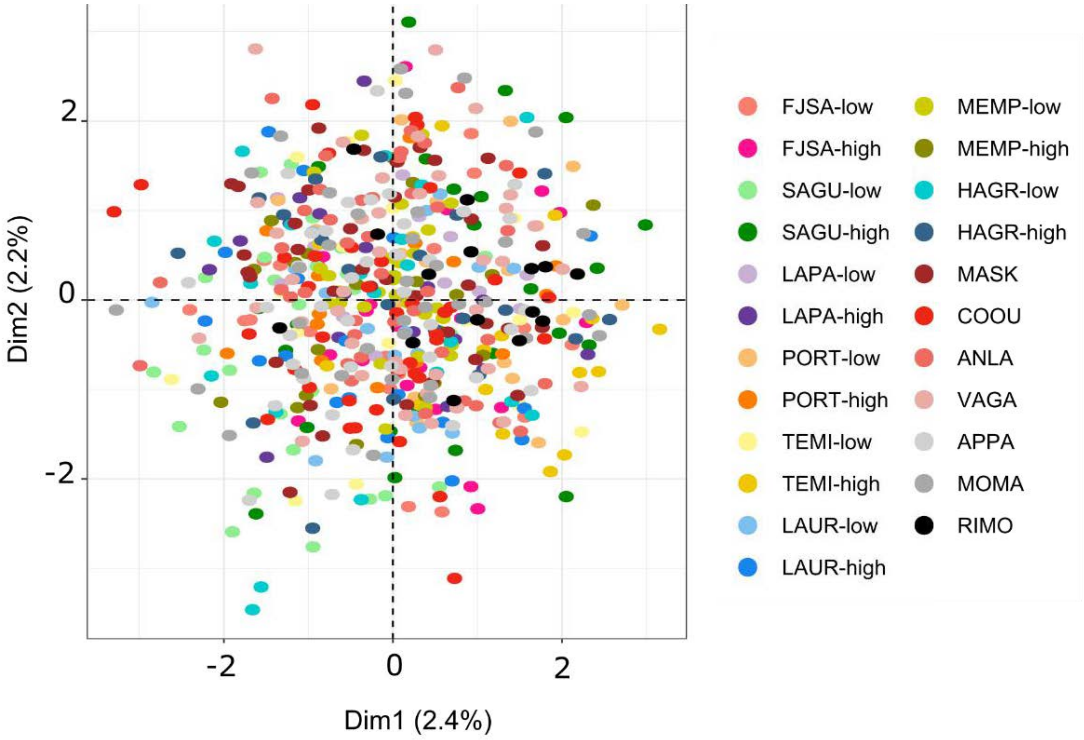

Figure 3.2: Principal Component Analysis (PCA) for 707 SNPs in all sampled individuals colored according to their population designated by codes listed in **Erreur ! Source du renvoi introuvable.**.

3.4.3 Local adaptation

3.4.3.1 Outliers approach by pcadapt at both scales

Regarding outliers' detection, since the PCA's first two axes explain most of variation, we retain them to compute SNP coordinates and thus identify outlier SNPs. This method identified 19 candidates SNPs at all scales (*i.e.* local and provincial). **Erreur ! Source du renvoi introuvable.** summarizes numbers of candidates SNPs and associated genes discovered by the outliers' approach at both scales and details about SNPs positions and gene IDs can be found in supplementary Table S6.

3.4.3.2 GEA approach by RDA at provincial scale

Regarding environmental data available for GEA, the exploration of significance of variables by the stepwise procedure and removal of correlated predictors and variables led to the selection of seven explanatory variables (Supplementary Figure 3.6; Supplementary). The model of ordination of the genomic variation according to these variables is significant (p-value=0.0167) with an $_{adj}R^2$ of 0.2633, implying that the constrained ordination explains about 26% of the genomic variation. **Erreur ! Source du renvoi introuvable.** resumes the contribution and significance of each e xplanatory variables to this ordination model. Furthermore, procedure of detection of candidates lead to the identification of 40 genomic positions candidate to selection and Supplementary Figure 3.7 presents the results of redundancy analysis of the adaptively enriched space based on these positions.

3.4.3.3 DAPC at local scale

Scatter plots of individuals among each pair along the calculated discriminant function show that they systematically segregate according to their position on the gradient (Figure 3.3). However, SNPs contributing the most (at least 1%) to discrimination among pairs are not the same across the pairs (supplementary Table S6).

3.4.3.4 Adaptive enriched space

Overall, 107 unique candidate SNPs were identified by at least one of the three methods explored here. **Erreur ! Source du renvoi introuvable.** resumes the results of the RDA analysis carried on t hese specific SNPs and the environmental variables selected during the previous RDA procedure while Figure 3.4 represents the projection of populations, candidate SNPs and environmental variables in this adaptively enriched RDA space.

Table 3.1: Table of redundancy analysis results for the neutral SNPs dataset. Codes of variables as well as units are provided in parenthesis. For "vegetation cover category" see supplementary Table 3.6 for details on the values taken by this variable.

Neutral ordination model (p-value = 0.0167) $_{adj}R^2$ = 0.2633948					
	Df	Variance	F	Pr(>F)	Proportion of variance
Average annual precipitation (avg_ann_prec; mm)	1	154.9	3.2764	0.0214 *	10.97
Average annual minimal temperature (avg_min_temp; °C)	1	94	1.9883	0.0967 ▪	6.66
Standard deviation annual wind speed (sd_ann_wind; $m.s^{-1}$)	1	134.58	2.8467	0.0354 *	9.53
Vegetation cover category (MYBB; BSME; YBF; ML)	3	255.24	1.7996	0.0601 ▪	18.07
Clay cation exchange capacity (CEC.clay; $cmolc.kg^{-1}$)	1	91.58	1.9370	0.1025	6.48
Elevation (elev; m)	1	29.16	0.6168	0.6663	2.06
Average annual solar radiation (avg_ann_srad; $kJ.m^{-2}.day^{-1}$)	1	37.94	0.8026	0.4644	2.69

Table 3.2: Table of redundancy analysis results for the candidate SNPs dataset. Codes of variables as well as units are provided in parenthesis. For "vegetation category" Supplementary Table 3.6 for details on the values taken by this variable (in parenthesis).

Adaptively enriched ordination model (p-value = 0.0075) $_{adj}R^2$ = 0.3069791					
	Df	Variance	F	Pr(>F)	Proportion of variance
Average annual precipitation (avg_ann_prec; mm)	1	24.439	3.6253	0.0194 *	11.42
Average annual minimal temperature (avg_min_temp; °C)	1	16.439	2.4371	0.0669 ▪	7.68
Standard deviation annual wind speed (sd_ann_wind; $m.s^{-1}$)	1	21.232	3.1496	0.0312 *	9.92
Vegetation category (MYBB; BSME; YBF; ML)	3	39.556	1.9559	0.0591 ▪	18.48
Clay cation exchange capacity (CEC.clay; $cmolc.kg^{-1}$)	1	13.684	2.0300	0.0917 ▪	6.39
Elevation (elev; m)	1	3.248	0.4818	0.8008	1.52
Average annual solar radiation (avg_ann_srad; $kJ.m^{-2}.day^{-1}$)	1	7.777	1.1536	0.2798	3.63

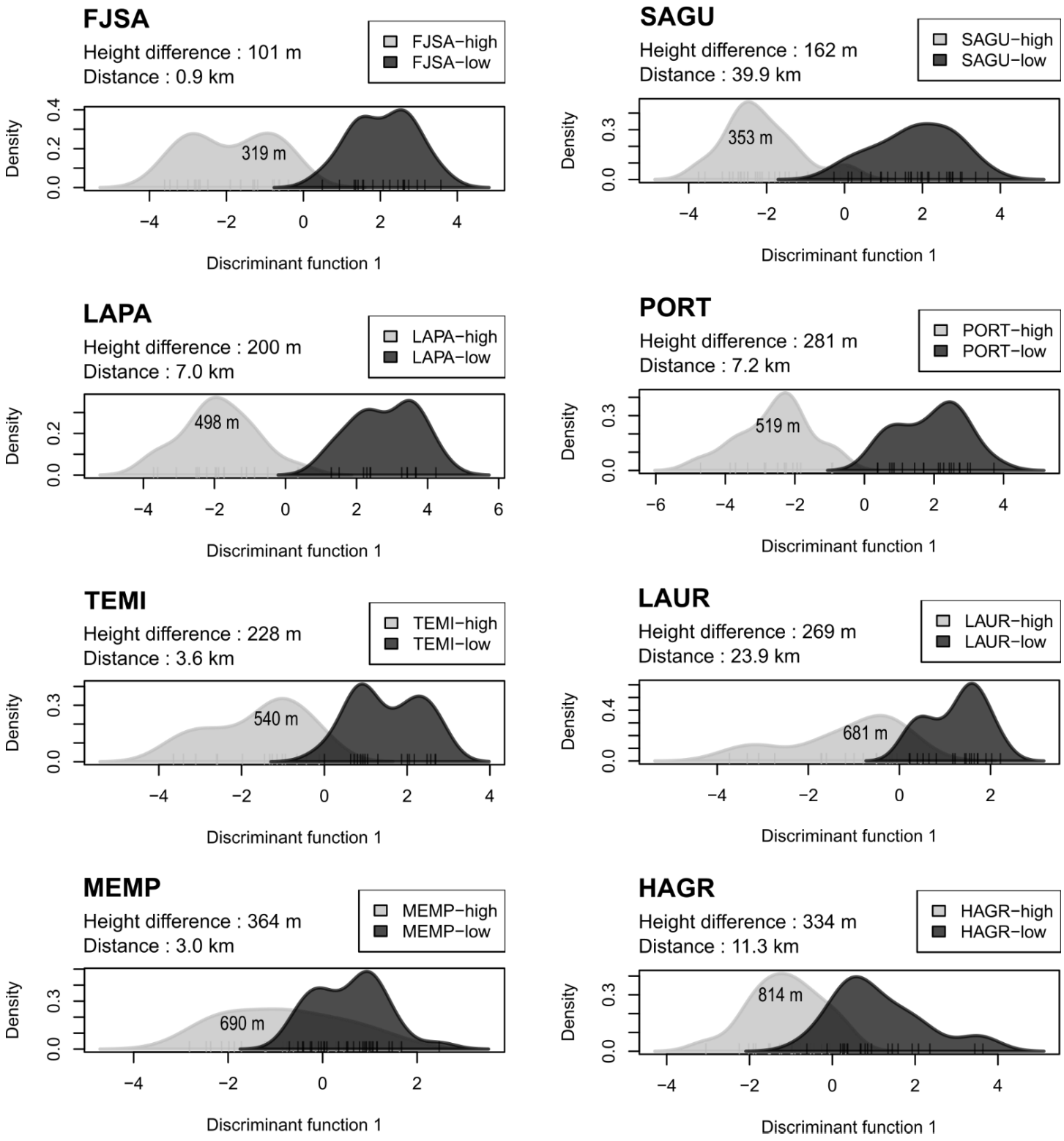

Figure 3.3: Scatter plots of populations on elevation gradients along one discriminant axis calculated by DAPC. Geographic distance and elevation variation are specified next to populations pairs' codes. Populations pairs are sorted from lowest to highest elevation of the 'high' population of the pair (light grey shade).

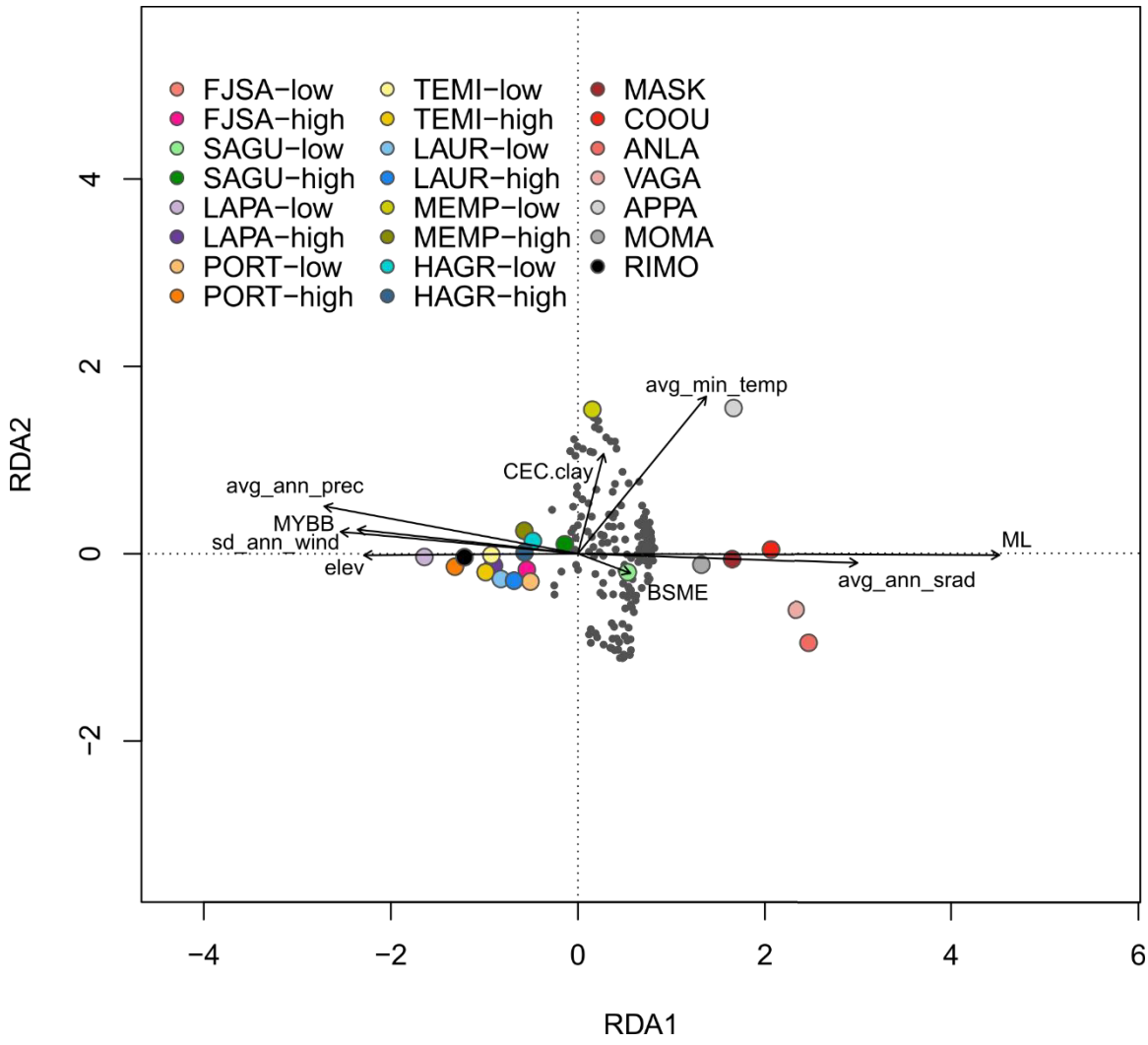

Figure 3.4: Projection of populations, loci and environmental variables into the adaptively enriched genetic space. Candidate loci are represented by dark grey spots and populations by colored dots.

3.5 Discussion

In this study, we investigated spatial patterns of genomic variation distribution among 23 populations of *A. saccharum* sampled in Quebec province (Canada) using a set of 707 SNPs generated through GBS (Elshire et al., 2011). We brought evidence for a homogenized distribution of genomic variation among populations, implying extensive gene flow across the province. The low populations differentiation indicates that they capture similar neutral genetic variation. This feature suggests that our data set is well suited for the detection of molecular imprints of

selection using outlier and GEA analyses (Lotterhos & Whitlock, 2015). Indeed, despite high gene flow among populations, we were able to identify 107 genomic positions, and 101 associated genes, candidate to selection at both provincial and local scales (Supplementary Table S6). Projection of these positions in an adaptively enriched RDA space allowed to identify association between genomic positions and environmental variables. Furthermore, high genetic discrimination among populations pairs situated on elevational gradients suggests local patterns of divergent selection linked to contrasted environments within small geographic scales.

3.5.1 Genetic diversity and gene flow

When considering all populations of *A. saccharum*, average Ho ($\overline{H_O}$ = 0.036) does not differ significantly from average He ($\overline{H_E}$ = 0.037). This contrasts with the mean inbreeding coefficient per population F_{IS} per population ($\overline{F_{IS}}$ = 0.055), which suggests a slight excess of homozygotes. However, tests for Hardy-Weinberg equilibrium revealed that most loci within populations were not in equilibrium, which may have influenced the F_{IS} estimates. Overall, levels of genetic diversity observed with genomic variants is consistent with previous studies based on microsatellites (Graignic 2014, 2016, 2018, Vargas, 2015, Jackson 2021). Research for genetic clustering brought evidence for high genetic admixture among populations (Supplementary Figure 3.5) and low genetic differentiation at individual (Figure 3.2) and populations levels for the provincial dataset ($\overline{F_{ST}} = 0.0226$; s.e. = 0.013; Supplementary). Therefore, our results suggest high gene flow among populations and confirm our first expectation based on past studies of genetic structure in the species (Perry and Knowles 1989; Gunter et al. 2000; Graignic, Tremblay, and Bergeron 2018). Furthermore, no isolation by distance was detected, supporting that populations are connected by gene flow. Regarding the genetic structure shaped by past demographic events, Vargas-Rodriguez et al. (2015) using microsatellites, identified a genetic signature of recolonization from LGM refugia in sugar maple. Similar genetic structuring due to historical demographic processes has been reported in other northeastern American tree species (Hewitt, 2004; Jaramillo-Correa et al., 2009). In our study, cross-validation error rates from the Admixture analysis indicated the presence of a single ancestral population. However, the frequencies of genetic clusters varied

across populations (Supplementary Figure 3.5, Supplementary Figure 3.8). This spatial variation in genetic cluster frequencies does not align with the expectation for a truly homogeneous ancestral population (Gravel, 2012). If all individuals originated from a single ancestral source, the distribution of genetic clusters for K = 2 should be relatively uniform across individuals. Instead, we found that for K = 2, 56% of our samples were predominantly composed of a single genetic cluster (*i.e.* Q > 0.99 for one cluster or the other). This non-uniform distribution of genetic clusters suggests that the populations did not originate from a single ancestral population but rather experienced demographic events that led to the formation of distinct genetic groups, later homogenized by extensive gene flow. Consequently, we propose that high levels of gene flow may have erased nuclear genetic signatures of past demographic events in sugar maple. A similar scenario has been suggested to explain the weak genetic structure observed in *Fagus sylvatica* in Europe (Pluess et al., 2016) and two hickory species in North America (Bemmels & Dick, 2018). Although no clear genetic structure attributable to past demographic events was detected with clustering approaches, alternative model-based methods explicitly designed to test demographic hypotheses, such as Approximate Bayesian Computation frameworks (e.g. DIYABC), coalescent-based migration models (e.g. Migrate-n), or Bayesian estimators of recent gene flow (e.g. BayesAss), could provide additional insights into the timing and magnitude of historical versus contemporary gene flow in sugar maple populations.

3.5.2 Local adaptation at the provincial scale

Despite high levels of gene flow, the distribution of genomic variation among *A. saccharum* populations reveals signals of local adaptation. We identified candidate loci under selection and their associated genes (Supplementary Table S6). Adaptive genomic variation detected across all methods (*i.e.* GEA, outliers and DAPC) showed a significant correlation with average annual precipitation and the standard deviation of wind speed. Additionally, weaker but near-significant correlations were found with minimum temperature, vegetation cover category, and clay cation exchange capacity (**Erreur ! Source du renvoi introuvable.**; Figure 3.4). Interestingly, when c onsidering only the candidate loci identified by GEA (n=40), the significance patterns of

explanatory variables in the ordination model differed (Supplementary Figure 3.7). In a partial adaptive model based solely on GEA candidates, average annual solar radiation was significantly correlated with genomic variation, whereas this correlation was absent when considering candidates identified by all methods. This difference highlights the variability in results among local adaptation detection methods and underscores the importance of using complementary approaches to gain a more comprehensive understanding of the local adaptation of natural populations (Forester et al., 2017).

Correlations between adaptive genomic variation and environmental variables align with previous findings from common garden experiments, which identified signatures of local adaptation to minimum temperature, precipitation levels, soil cation exchange capacity, and solar radiation (Carteron et al., 2020; Collins et al., 2018; Guo et al., 2020; Solarik et al., 2016). However, despite these correlations, our dataset does not provide evidence of local adaptation along longitudinal or latitudinal gradients (Guo et al., 2023; Rowe, 1972). Neither latitude nor longitude were retained in the variable selection procedure for GEA analysis, indicating that they do not represent the primary explanatory factors driving genomic variation in our study. Additionally, population projections in RDA space do not exhibit spatial structuring according to latitude or longitude (Figure 3.4, Supplementary Figure 3.7), further suggesting that local adaptation along these gradients was not captured. This is likely due to our sampling design, which was concentrated within the province of Quebec rather than spanning the species' entire geographic range.

Beyond climatic variables, spatial variation in local disturbance regimes may also contribute to the observed patterns of adaptive genomic variation. Across Quebec, *A. saccharum* populations experience heterogeneous disturbance histories driven by windthrow events, ice storms, insect outbreaks, forest management practices, and stand age dynamics, all of which can vary markedly over relatively short spatial scales. Such disturbances can modify selective pressures indirectly by altering canopy openness, microclimatic conditions, soil properties, and competitive environments, thereby influencing regeneration dynamics and survival. In particular, wind-related disturbances may interact with the observed correlations between genomic variation and

wind speed variability, suggesting that adaptation may reflect not only tolerance to climatic conditions but also responses to disturbance-mediated selection. Although disturbance regimes were not explicitly quantified in this study, their potential role highlights the complexity of selective environments experienced by sugar maple populations and suggests that future studies integrating disturbance history and genomic data would provide valuable insights into the mechanisms underlying local adaptation.

Therefore, our findings align with previous research highlighting that local adaptation is often driven by complex environmental factors beyond simple latitudinal and longitudinal gradients (Savolainen et al., 2013). Indeed, population projections within the adaptively enriched RDA space (Figure 3.4) reveal strong associations between adaptive genomic variation and elevation, wind speed, and precipitation. These findings underscore the influence of population pairs located in environmentally contrasting regions due to elevation differences (**Erreur ! Source du r envoi introuvable.**). Overall, our results suggest that selective pressures associated with elevation gradients play a major role in shaping local adaptation, with genomic variation reflecting adaptation to heterogeneous environmental conditions characterized by both atmospheric and soil factors (**Erreur ! Source du renvoi introuvable.**). This aligns with findings from Halbritter et al. (2015), who demonstrated that adaptation at range edges is often more pronounced along elevation gradients than along latitudinal ones.

3.5.3 Divergent selection

Our analysis revealed a systematic differentiation in allelic frequencies between populations forming elevation gradient pairs (Figure 3.3). Given their geographical proximity and the low pairwise F_{ST} values within pairs (Supplementary), this genetic differentiation suggests the influence of natural selection on allelic frequencies. Furthermore, this suggests that selection exerts a greater influence on allelic frequencies than migration within these pairs. This pattern challenges the classical expectation that gene flow counteracts the effects of selection (Kremer

et al., 2012) and instead aligns with empirical evidence of population or species divergence despite extensive gene flow (Bolte et al., 2022; Latta, 2004).

The observed genetic differentiation along elevation gradients suggests that contrasting environmental conditions at either end of the gradient impose divergent selective pressures. Elevation gradients generate sharp microclimatic variation over short geographic distances, including differences in temperature regimes, frost frequency, wind exposure, snow cover duration, and growing season length. Such fine-scale environmental heterogeneity can create strong selective mosaics that are not captured by broader macroclimatic gradients such as latitude or longitude. In this context, our results support the hypothesis that local adaptation in sugar maple driven by microclimatic variation associated with elevation and by large-scale macroclimatic patterns.

Elevation-driven selection has previously been documented in sugar maple by Ledig and Korbobo (1983), who identified physiological trait differences, such as including photosynthesis, respiration, and specific leaf weight, linked to local adaptation. Similarly, divergent selection along an elevational gradient has been reported in *Populus trichocarpa* (Zhang et al., 2019). To investigate local adaptation along elevation gradients, we employed two complementary approaches: a multivariate clustering analysis (DAPC) and an outlier detection method (pcadapt). Across the eight studied population pairs, there was minimal overlap in candidate SNPs identified by these methods. This finding underscores the importance of integrating multiple approaches when detecting local adaptation. Moreover, even among the SNPs identified by both methods, the loci putatively involved in adaptation varied between pairs (Supplementary Table S6). This suggests that despite experiencing similar environmental contrasts across the province, different populations may be subject to selection on distinct genomic regions.

Taken together, these results indicate that sugar maple populations respond to fine-scale environmental heterogeneity through multiple, potentially redundant, adaptive pathways. This pattern is consistent with a model of microclimate-driven adaptation, where local selective pressures vary over short spatial scales and shape genomic variation independently across

populations. Such microclimatic adaptation likely contributes to the high adaptive potential and phenotypic plasticity of sugar maple, as previously documented in common garden and physiological studies (Guo et al., 2023; Nolet et al., 2008).

3.5.4 Implication for conservation strategy and tree breeding programs

Ultimately, our study provides evidence for local adaptation despite high levels of gene flow in natural populations of a wind-pollinated tree species. Moreover, the genomic variants associated with local adaptation varied widely across the province, underscoring the crucial role of standing genetic variation as a reservoir for adaptation to environmental conditions and highlighting the polygenic nature of climate-related traits (Ahrens et al., 2019). Among the identified adaptive variants, two were located in close proximity (≤ 900 bp) to protein-coding genes with functional annotations in the Ensembl Plants database (Bolser et al., 2017). These genes (AT5G40990 and AT2G02990) have been described in other plant species (*e.g.*, *Arabidopsis thaliana*, *Brassica rapa*) and are involved in enzyme synthesis. AT5G40990 encodes a GDSL lipase, a hydrolytic enzyme with multifunctional properties, including substrate specificity and regiospecificity, while AT2G02990 encodes a ribonuclease. However, these genes are widely conserved and do not provide direct insights into functions specifically involved in climate adaptation. The remaining candidate variants were in poorly characterized regions of the sugar maple genome, emphasizing the limited genomic resources available to fully elucidate the genetic basis of adaptation in this species. Overall, our study identifies genomic regions associated with local adaptation to key environmental variables, providing a valuable resource for future research on assisted gene flow (Aitken, Yeaman, Holliday, Wang, & Curtis-McLane, 2008) and adaptive breeding programs for sugar maple.

3.6 Conclusion and perspectives

In this study, we used newly generated GBS data to investigate the genetic differentiation and local adaptation of *Acer saccharum* across Quebec. Our results revealed no evidence of population structure within the sampled region, supporting the idea that the species' wind-mediated pollination facilitates extensive gene flow over large geographic scales. Despite this high gene flow, we identified signatures of local adaptation to environmental variation, with selective pressures acting on diverse genes, suggesting divergent selection among populations. This indicates that populations have adapted to their microclimates and developed localized strategies to cope with environmental pressures. While previous studies have examined local adaptation in sugar maple through physiological trait measurements in common garden experiments (Gunderson et al., 2000; Guo et al., 2023; McCarragher et al., 2011; Ren et al., 2021), our study is among the first to investigate the genomic basis of local adaptation in this species. This provides a valuable foundation for future research on genotype selection for assisted migration programs. However, further studies are necessary to confidently characterize the genetic mechanisms underlying local adaptation to climate in sugar maple. Indeed, our detection of local adaptation relied on a multi-locus approach, which presents inherent challenges. When using multi-locus analyses, significant associations with climate may arise due to covariance in allele frequencies across numerous loci with small effects, rather than through frequency shifts at a few loci with larger effects (Latta, 1998, 2004), as observed in *Pinus strobus* (Rajora et al 2016). This mode of adaptation via multi-locus covariance is expected under conditions of high gene flow and recent selection (Le Corre & Kremer, 2012) , the former being observed and the latter being suspected in our study. To refine our understanding of the genomic basis of local adaptation in this species, combining multi-locus analyses with targeted single-locus approaches, that focus on genes known to influence adaptive physiological traits, could be particularly informative, as demonstrated by Nadeau et al. (2016) for *Pinus strobus*. However, such analyses require an expansion of available genomic resources, particularly the functional annotation of genes associated with adaptive traits in sugar maple. Approaches such as Quantitative Trait Loci (QTL) mapping (Sewell & Neale, 2000) could be essential in bridging this gap.

3.7 Acknowledgements

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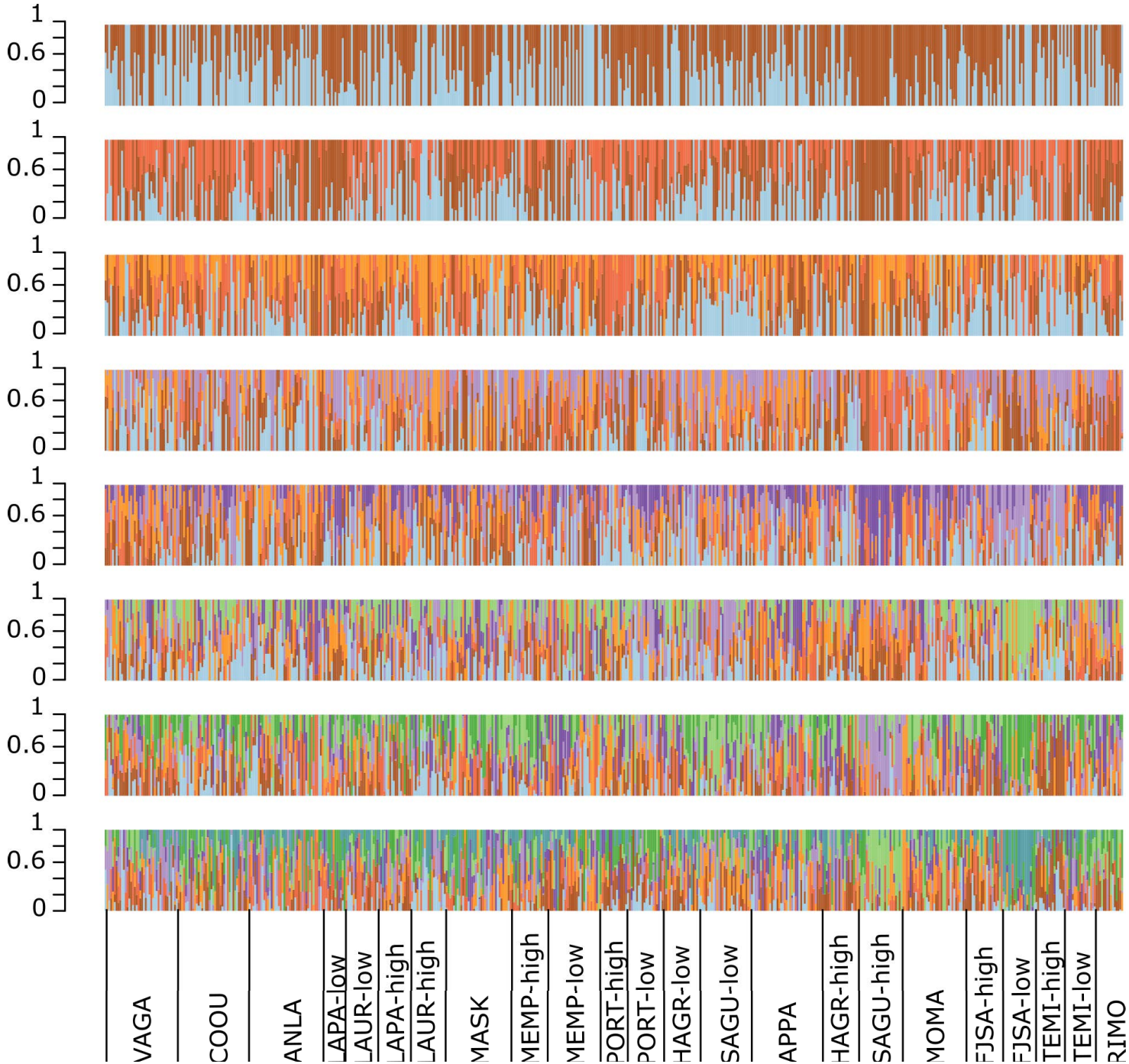
Supplementary Table 3.3: Genotyping error rates for four different MAF filtering values.

ID	MAF=0001			MAF=0005			MAF=001			MAF=005		
	N genotypes	N different	diff_rate	N genotypes	N different	diff_rate	N genotypes	N different	diff_rate	N genotypes	N different	diff_rate
MOMA01.23		326	0.0455		206	0.0502		157	0.0538		85	0.0604
MOMA01.24	7164	418	0.0583	4103	248	0.0604	2919	187	0.0641	1408	95	0.0675
RIMO01.20		327	0.0456		187	0.0456		148	0.0507		66	0.0469
MEAN			**0.0498**			**0.0521**			**0.0562**			**0.0582**

Supplementary Table 3.4: Metadata and genetic statistics for populations available for analyses, that is after losses in laboratory and bioinformatic procedures. N column refers to number of individuals per population after bioinformatic treatments.

Code	Pairs	N	mean N reads	mean N SNPs	mean depth	*Ho*	*He*	F_{IS}
FJSA-low	FJSA	18	2.7×10^6	674.9	119.5	0.032	0.031	-0.012
FJSA-high		20	3.0×10^6	690.9	138.2	0.034	0.036	0.089
SAGU-low	SAGU	28	3.3×10^6	683.9	129.3	0.035	0.038	0.084
SAGU-high		24	2.4×10^6	686.6	94.1	0.035	0.036	0.046
LAPA-low	LAPA	12	1.5×10^6	660.5	60.6	0.033	0.031	0.001
LAPA-high		18	1.6×10^6	651.3	63.3	0.034	0.035	0.041
PORT-low	PORT	20	1.9×10^6	680.3	81.1	0.037	0.038	0.049
PORT-high		15	2.0×10^6	657.9	88.8	0.037	0.037	0.027
TEMI-low	TEMI	17	2.4×10^6	676.5	96.2	0.036	0.039	0.100
TEMI-high		16	4.0×10^6	686.3	157.7	0.032	0.031	0.019
LAUR-low	LAUR	18	1.2×10^6	673.9	47.2	0.040	0.040	0.023
LAUR-high		19	2.0×10^6	667.8	79.0	0.038	0.039	0.034
MEMP-low	MEMP	29	9.5×10^5	590.1	47.2	0.034	0.036	0.076
MEMP-high		20	1.8×10^6	686.6	76.1	0.040	0.041	0.042
HAGR-low	HAGR	20	3.1×10^6	693.0	127.4	0.032	0.034	0.074
HAGR-high		20	3.6×10^6	689.8	153.8	0.032	0.036	0.118
MASK		36	3.0×10^6	690.4	122.7	0.033	0.037	0.118
COOU		39	1.5×10^6	686.9	61.9	0.040	0.040	0.032
ANLA		41	1.9×10^6	689.9	74.7	0.037	0.039	0.070
VAGA		40	2.0×10^6	692.0	78.9	0.036	0.039	0.097
APPA		39	2.0×10^6	666.9	78.8	0.039	0.039	0.022
MOMA		35	2.6×10^6	685.7	97.6	0.037	0.040	0.078
RIMO		15	1.5×10^6	685.1	68.3	0.037	0.037	0.030



K	CV error
1	0.114
2	0.121
3	0.126
4	0.128
5	0.129
6	0.132
7	0.136
8	0.136
9	0.139
10	0.141
11	0.141
12	0.143
13	0.144
14	0.146
15	0.147
16	0.150
17	0.150
18	0.152
21	0.153
19	0.154
20	0.154
22	0.156
23	0.157

Supplementary Figure 3.5 : Individual composition in genetic clusters found by Admixture for 2<K<9 on the 707 SNPs. Each bar represents an individual and these are sorted according to their longitude coordinates (west to east). Populations are designated by codes listed in **Erreur ! Source du renvoi introuvable.**. The CV error values for each value of K are listed in the adjacent table.

Supplementary Table 3.5: Pairwise population FST values (down) and geographic distances in km (up). Populations are designated by codes listed in Supplementary Table 3.4. Values circled by dashed lines correspond to FST and geographic distances within pairs of populations.

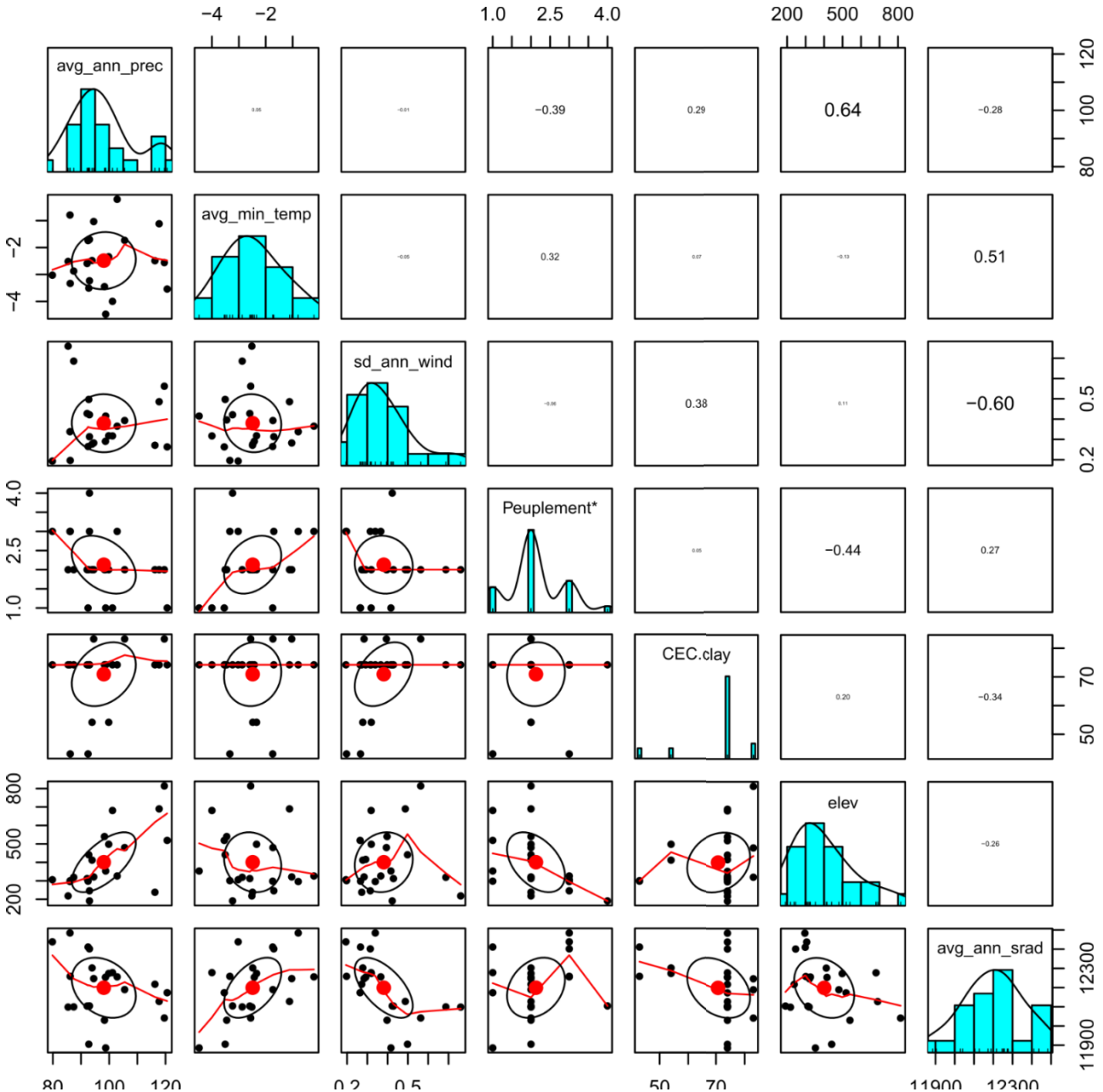
	FJSA-low	FJSA-high	SAGU-low	SAGU-high	LAPA-low	LAPA-high	PORT-low	PORT-high	TEMI-low	TEMI-high	LAUR-low	LAUR-high	MEMP-low	MEMP-high	HAGR-low	HAGR-high	MASK	COOU	ANLA	VAGA	APPA	MOMA	RIMO
FJSA-low	-	0.9	85.7	77.0	412.8	409.7	178.4	185.5	99.4	96.1	405.7	385.5	350.3	353.3	319.1	310.4	292.4	531.9	433.2	483.4	269.0	144.0	143.2
FJSA-high	0.036	-	85.0	76.1	412.8	409.8	178.5	185.6	100.3	97.1	405.7	385.5	350.7	353.7	319.7	311.0	292.5	531.9	432.9	483.2	269.5	144.7	143.7
SAGU-low	0.043	0.019	-	39.9	360.4	358.5	144.6	151.1	170.5	166.9	352.6	330.4	338.0	341.0	325.9	319.4	248.9	474.6	363.4	412.5	274.9	170.2	227.3
SAGU-high	0.063	0.042	0.053	-	400.1	398.2	182.9	189.7	174.2	170.7	392.2	369.9	374.5	377.5	358.1	351.0	288.7	513.7	400.0	448.5	306.9	194.1	207.2
LAPA-low	0.062	0.030	0.044	0.047	-	7.0	236.1	229.2	418.9	416.8	8.4	32.3	190.2	190.2	262.7	269.8	123.4	121.5	114.6	144.9	257.9	326.3	537.9
LAPA-high	0.041	0.018	0.017	0.053	0.035	-	232.6	225.6	414.4	412.3	11.5	33.7	183.2	183.2	255.8	263.0	119.3	126.0	121.0	152.0	251.5	321.4	533.9
PORT-low	0.049	0.032	0.038	0.052	0.032	0.031	-	7.2	189.7	187.1	229.4	210.6	195.4	198.4	197.1	193.3	114.2	356.7	274.6	325.3	149.7	111.6	302.8
PORT-high	0.050	0.024	0.037	0.050	0.028	0.032	0.036	-	195.7	193.0	222.5	203.9	189.8	192.7	193.6	190.2	107.1	349.9	269.0	319.7	147.0	115.6	309.5
TEMI-low	0.027	0.011	0.018	0.032	0.017	0.010	0.015	0.023	-	3.6	413.1	396.6	311.5	314.3	261.8	251.4	295.8	540.4	464.1	514.9	217.4	96.8	126.6
TEMI-high	0.054	0.021	0.045	0.066	0.061	0.034	0.037	0.045	0.027	-	410.9	394.3	310.8	313.6	261.8	251.5	293.6	538.3	461.3	512.1	217.1	95.5	127.5
LAUR-low	0.031	0.014	0.027	0.031	0.021	0.019	0.017	0.012	0.003	0.035	-	23.9	190.4	190.6	261.8	268.7	117.4	127.5	110.6	143.5	255.4	321.0	531.5
LAUR-high	0.046	0.031	0.030	0.061	0.044	0.020	0.034	0.039	0.019	0.050	0.019	-	192.3	192.8	260.0	266.0	101.6	146.5	103.1	142.7	248.9	306.1	513.4
MEMP-low	0.027	0.014	0.014	0.032	0.017	0.005	0.022	0.018	0.005	0.017	0.009	0.021	-	3.0	77.5	87.1	139.3	289.3	295.2	333.5	97.7	217.0	438.0
MEMP-high	0.042	0.014	0.022	0.035	0.020	0.027	0.031	0.024	0.003	0.034	0.012	0.030	0.009	-	78.8	88.6	141.3	288.5	295.8	333.8	100.2	219.9	440.8
HAGR-low	0.032	0.015	0.010	0.051	0.038	-0.001	0.036	0.025	0.009	0.031	0.018	0.021	0.004	0.018	-	11.3	185.8	366.3	360.9	402.7	51.2	176.0	385.7
HAGR-high	0.033	0.009	0.024	0.040	0.031	0.011	0.029	0.024	0.009	0.026	0.010	0.029	0.006	0.020	0.011	-	188.7	375.1	366.3	408.7	44.7	166.9	375.0
MASK	0.031	0.013	0.019	0.038	0.017	0.007	0.026	0.017	0.007	0.031	0.015	0.019	0.002	0.013	0.004	0.011	-	244.8	186.5	234.2	161.0	204.5	414.6
COOU	0.031	0.020	0.021	0.039	0.026	0.010	0.027	0.017	0.007	0.037	0.008	0.019	0.010	0.020	0.012	0.015	0.009	-	143.2	127.6	370.6	447.4	659.1
ANLA	0.036	0.014	0.020	0.038	0.027	0.017	0.025	0.017	0.008	0.031	0.008	0.023	0.010	0.019	0.018	0.013	0.009	0.010	-	50.9	345.1	381.9	570.9
VAGA	0.034	0.015	0.020	0.039	0.031	0.008	0.020	0.024	0.008	0.025	0.013	0.018	0.007	0.020	0.008	0.012	0.007	0.010	0.009	-	390.3	432.1	621.6
APPA	0.029	0.011	0.019	0.030	0.015	0.013	0.021	0.023	0.004	0.028	0.008	0.024	0.004	0.007	0.010	0.009	0.005	0.013	0.009	0.013	-	127.3	343.2
MOMA	0.041	0.011	0.017	0.032	0.025	0.015	0.024	0.018	0.007	0.030	0.012	0.024	0.008	0.016	0.016	0.010	0.011	0.014	0.012	0.012	0.011	-	222.8
RIMO	0.048	0.016	0.032	0.027	0.013	0.026	0.023	0.020	0.004	0.034	0.012	0.034	0.002	0.015	0.023	0.017	0.017	0.014	0.014	0.023	0.012	0.012	-

Supplementary Table 3.6 : Values for the selected environmental variables at the population level and summary of number of candidate SNPs and associated genes found at both scale by the different methods. In the 'vegetation cover category' column: MYBB stands for 'Maple bush with yellow birch or beech', BSME for 'Black spruce with moss or ericaceous', YBF for 'Yellow birch grove with fir or fir grove with yellow birch' and ML for 'Maple bush with linden'. The (●) next to a genes' number indicates that one the genes associated with candidate SNPs is described in the Ensembl Plants gene database (Bolser et al., 2017).

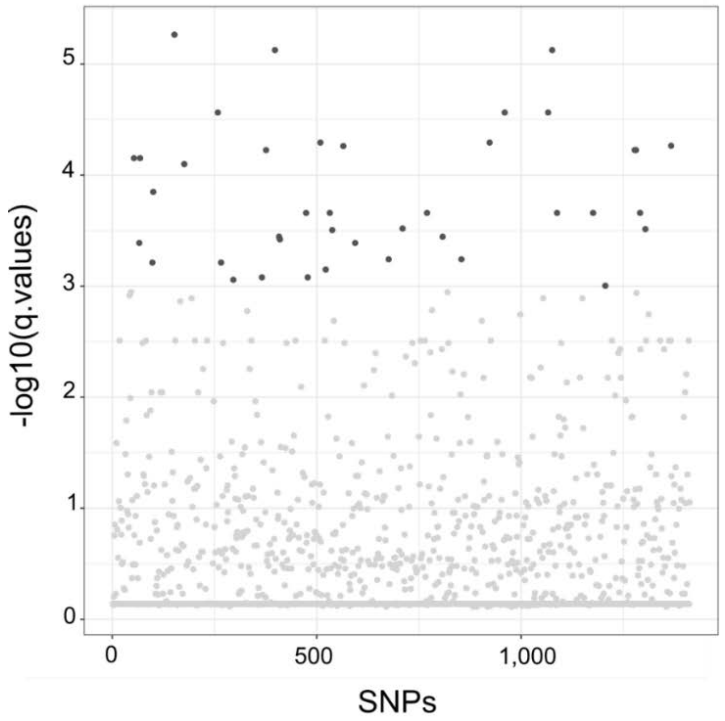
	Environmental variables							Local (pairs)				Global			
								Pcadapt		DAPC		Pcadapt		RDA	
Population	precipitation (avg; mm)	minimal temperature (avg; °C)	wind speed (sd; $m.s^{-1}$)	vegetation cover category	CEC.clay ($cmolc.kg^{-1}$)	solar radiation (avg; $kJ.m^{-2}.day^{-1}$)	Elevation (m)	SNPs	Genes	SNPs	Genes	SNPs	Genes	SNPs	Genes
FJSA-low	85.42	-2.52	0.76	MYBB	74	1,2097.8	218	NA	NA	13	12				
FJSA-high	87.42	-2.88	0.69	MYBB	74	1,2098.8	319								
SAGU-low	93.00	-3.23	0.42	BSME	74	1,2103.8	191	2	2●	14	17				
SAGU-high	98.75	-4.48	0.42	YBF	74	1,1885.5	353								
LAPA-low	92.50	-1.74	0.27	YBF	43	1,2409.8	298	4	4	9	8				
LAPA-high	99.83	-2.34	0.32	MYBB	54	1,2272.7	498								
PORT-low	116.25	-2.49	0.27	MYBB	74	1,2216.5	238	5	5	14	14●				
PORT-high	120.58	-3.54	0.26	YBF	74	1,2172.8	519								
TEMI-low	92.17	-2.59	0.43	MYBB	74	1,2103.2	312	2	2	14	13				
TEMI-high	98.33	-3.45	0.40	MYBB	74	1,2029.6	540								
LAUR-low	93.92	-2.48	0.28	MYBB	54	1,2301.4	412	NA	NA	6	6				
LAUR-high	101.17	-4.00	0.32	YBF	74	1,2277.1	681					2	2	40	37
MEMP-low	102.83	-0.21	0.37	ML	74	1,2254.9	326	2	1	8	8				
MEMP-high	117.75	-1.12	0.49	MYBB	74	1,2127.0	690								
HAGR-low	105.50	-1.73	0.39	MYBB	83	1,2188.8	480	4	3	12	11				
HAGR-high	119.58	-2.56	0.56	MYBB	83	1,2040.9	814								
MASK	93.00	-1.70	0.31	ML	74	1,2400.6	246								
COOU	86.08	-0.79	0.34	ML	74	1,2483.1	296								
ANLA	86.17	-3.33	0.20	ML	43	1,2257.6	301								
VAGA	79.83	-3.03	0.19	ML	74	1,2437.0	306								
APPA	94.50	-1.03	0.28	MYBB	83	1,2246.0	321								
MOMA	98.67	-2.43	0.29	MYBB	74	1,2252.8	416								
RIMO	92.75	-3.51	0.50	MYBB	74	1,1904.9	441								

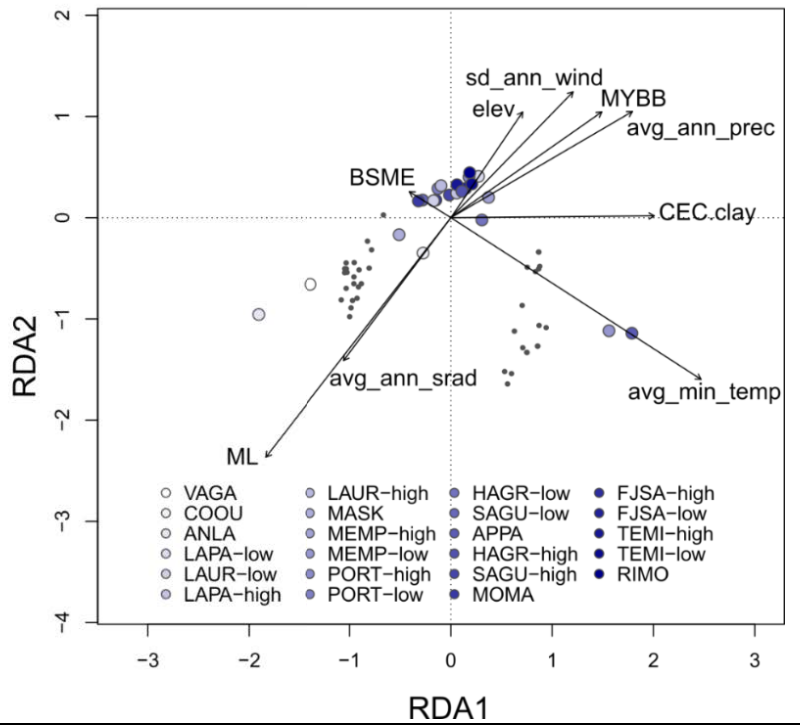
Supplementary Table 3.7 : VIF calculations for the selected variables in RDA models.

	VIF
Average annual precipitation (avg_ann_prec)	2.78
Average annual minimal temperature (avg_min_temp)	2.63
Standard deviation annual wind speed (sd_ann_wind)	2.38
Vegetation category (Peuplement)	
MYBB	2.76
ML	3.15
BSME	1.44
CEC.clay	1.41
Elevation (elev)	2.09
Average annual solar radiation (avg_ann_srad)	3.66



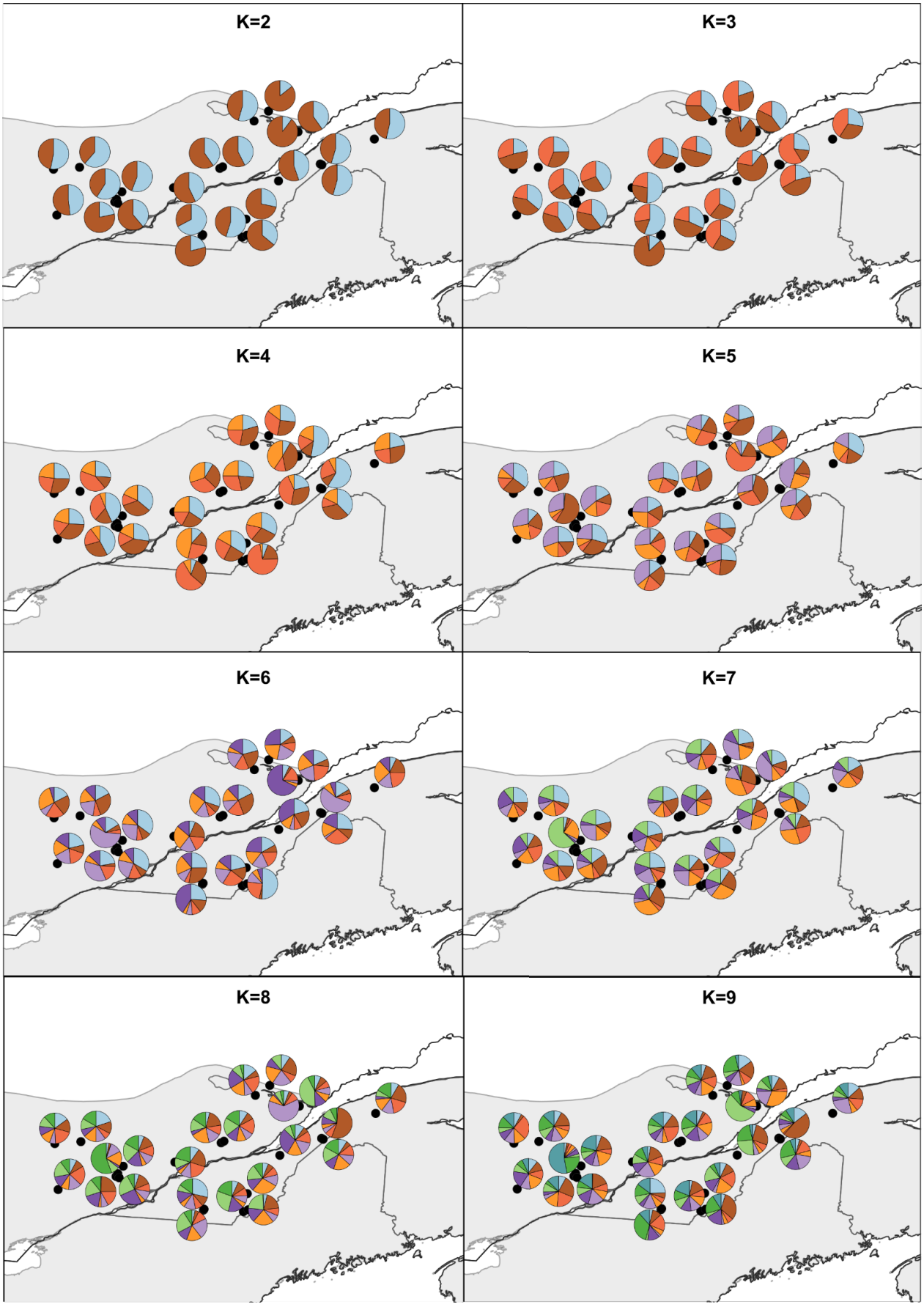
Supplementary Figure 3.6: Correlation matrix between selected variables.





RDA selected adaptively enriched genetic model (p-value = 0.0153) $_{adj}R^2$ = 0.295883					
	Df	Variance	F	Pr(>F)	Proportion of variance
Average annual precipitation (avg_ann_prec; mm)	1	3.0002	2.3435	0.0527 ▪	7.5
Average annual minimal temperature (avg_min_temp; °C)	1	5.6142	4.3854	0.0031 **	14.03
Standard deviation annual wind speed (sd_ann_wind; $m.s^{-1}$)	1	2.4522	1.9155	0.0983 ▪	6.13
Vegetation category (MYBB; BSME; YBF; ML)	3	5.8722	1.5290	0.1893	14.68
CEC.clay ($cmolc.kg^{-1}$)	1	2.6403	2.0624	0.0834 ▪	6.6
Elevation (elev; m)	1	0.5628	0.4396	0.8206	1.407
Average annual solar radiation (avg_ann_srad; $kJ.m^{-2}.day^{-1}$)	1	3.2152	2.5115	0.0366 *	8.03

Supplementary Figure 3.7 : (up left) Manhattan plot of the GEA analysis using RDA. Loci with a q-value inferior to 10^{-3} are coloured in dark grey and were considered as candidates to selection. (up right) Projection of RDA candidates and environmental variables into the adaptively enriched genetic space. Candidate loci are colored in dark grey, and populations are coloured in shade of blues according to their longitude (white = west; dark blue = east). (bottom) Table of RDA results for the candidate SNPs dataset. Codes of variables as well as units are provided in parenthesis. For "vegetation category" see **Erreur ! Source du renvoi introuvable.** for details on the values taken b y this variable (in parenthesis).



Supplementary Figure 3.8 : Distribution maps of populations frequencies in genetic clusters (K) found by Admixture.

Conclusion générale

Dans le contexte actuel des changements globaux, la persistance des organismes dans de nouveaux environnements dépend de leur capacité d'adaptation (Nicotra et al., 2015; Thurman et al., 2020). Cette dernière repose sur divers mécanismes agissant à court terme, tels que l'acclimatation (*i.e.* la mise en place de mécanismes physiologiques ou épigénétiques permettant aux individus de persister dans leur milieu), ou à long terme, comme l'adaptation (*i.e.* l'augmentation de la fréquence de certains variants génétiques dans les populations au fil des générations sous l'effet de la sélection naturelle) (Benomar et al., 2016; Bussotti et al., 2015; Domingues et al., 2018). Dans le cas des forêts tempérées, l'étude du potentiel adaptatif (*i.e.* la propriété d'une entité biologique à répondre à la sélection naturelle par un changement évolutif, laquelle dépend de la quantité et de la distribution de la variation génétique héréditaire affectant les traits liés à l'aptitude biologique) des espèces arborées constitue une étape clé pour appréhender leur capacité d'adaptation à long terme face aux changements climatiques (Alberto et al., 2013; Milesi et al., 2024). Cette thèse avait pour objectif principal de discuter du potentiel adaptatif de l'érable à sucre (*Acer saccharum*) en tant qu'espèce emblématique des forêts tempérées de l'est de l'Amérique du Nord. L'approche utilisée pour discuter du potentiel adaptatif de cette espèce consistait à détecter les bases génétiques de l'adaptation locale au climat dans des populations naturelles. Cette approche, ayant fait ses preuves chez d'autres espèces arborées (Capblancq, Morin, et al., 2020; Martins et al., 2018; Pluess et al., 2016), se base sur le fait que le potentiel adaptatif des arbres dépend grandement de leur diversité génétique préexistante, c'est-à-dire de la diversité déjà en place, dans laquelle de nouvelles mutations peuvent apparaître et être sélectionnées (Sork et al., 2013). L'identification de la diversité génétique adaptative, c'est-à-dire issue d'un processus de sélection, constitue un premier pas vers la connaissance du potentiel adaptatif des populations (Kremer, 2000). Cependant, identifier la variation génétique associée à l'adaptation locale dans les populations naturelles nécessite de distinguer les effets relatifs des différents processus évolutifs influençant les fréquences alléliques (Rellstab et al., 2015). En effet, ces fréquences varient sous l'effet de quatre forces évolutives : la mutation, la dérive génétique, la sélection (e.g. adaptation locale) et la migration

(e.g. événements migratoires passés ou hybridation avec des espèces proches). L'érable à sucre fait partie de la série *Saccharodendron*, *i.e.* un ensemble de taxons désignés comme espèces, sous-espèces, variétés ou écotypes selon les nomenclatures (Davis, 2021; de Jong, 2002). Les membres de cette série taxonomique ayant la réputation de s'hybrider facilement (Jackson, 2020), les deux premiers chapitres de cette thèse se sont attelés à identifier les limites génétiques de ces taxons. Le troisième chapitre quant à lui discute des potentielles causes du patron de distribution de la variation génétique au sein de populations d'érable à sucre au Québec. Ainsi, les différents chapitres de cette thèse ont tout d'abord mis en évidence que l'érable à sucre, tel que décrit morphologiquement en 1785 (Marshall), correspond à une lignée monophylétique au sein de la série *Saccharodendron*. Deuxièmement, cette thèse apporte des preuves que l'érable à sucre est génétiquement distinct de son plus proche parent sympatrique, l'érable noir. Troisièmement, le dernier chapitre met en évidence une distribution homogène de la variation génétique au sein des populations d'érable à sucre au Québec, signe que d'importants flux de gènes ont brouillé les traces des migrations passées. Par ailleurs, ce dernier chapitre révèle la présence de signaux d'adaptation locale aux variations climatiques, malgré ces flux de gènes importants. Cette conclusion générale remet en perspective ces résultats au vu de la littérature existante afin de mettre en évidence les apports et les limites de la thèse. L'identification des différentes limites permet également de discuter de plusieurs perspectives de recherche afin d'exploiter plus avant ces résultats.

4.1 La série *Saccharodendron* : un ensemble d'érables à sucre

4.1.1 Continuum de spéciation et nomenclature

Le processus de spéciation peut être considéré à deux échelles temporelles. D'une part, à une échelle temporelle large (*i.e.* sur de longs temps évolutifs) la spéciation est perçue comme un événement discret, une frontière qui marque le passage des populations aux espèces. D'autre part, à une échelle temporelle plus fine, la spéciation désigne le processus qui génère les groupes distincts que nous reconnaissons finalement comme espèces. Cette deuxième vision a donné lieu

à la définition du continuum de spéciation qui regroupe les étapes successives menant des populations connectées à devenir deux espèces distinctes. Selon la définition donnée par Stankowski et Ravinet (2021), les différentes étapes regroupées dans le continuum de spéciation correspondent à différents degrés d'isolement reproducteur. La série *Saccharodendron* constitue un bon exemple de continuum de spéciation puisque les connaissances actuelles sur l'histoire évolutive de ce groupe supposent que ces taxons ont divergé assez récemment (Areces-Berazain et al., 2021; Gao et al., 2020). Cette divergence récente, additionnée de la proximité morphologique et des cas d'hybridation forcés et supposés entre certains membres de ce groupe, a mené par le passé à la considération que ces taxons constituaient une seule espèce, partageant une génétique commune mais présentant des variations morphologiques, désignées comme écotypes, variétés ou sous-espèces (de Jong, 2002; Kriebel, 1957; Skepner & Krane, 1998). Ce n'est que récemment qu'une étude basée sur des microsatellites a supposé que ces taxons présentent des pools génétiques distincts (Jackson et al., 2021). Dans la lignée de cette étude, cette thèse soutient que plusieurs concepts d'espèces sont respectés au sein de la série *Saccharodendron*. Dans un premier temps, étant donné que tous les échantillons récoltés pour cette thèse ont été assignés à un taxon selon leurs morphologies, cela implique que le concept morphologique d'espèces est respecté. Dans un deuxième temps, le chapitre un confirme que pour tous les taxons du groupe, à l'exception d'*A. skutchii*, les hypothèses d'espèces basées sur la morphologie correspondent à des lignées monophylétiques, ce qui confirme le respect du concept phylogénétique d'espèces. La monophylie du taxon *A. skutchii* avait déjà été démontrée par le passé (Areces-Berazain et al., 2021; Gao et al., 2020) et il est possible que des biais d'échantillonnage ou techniques aient affecté nos résultats. Enfin, le deuxième chapitre apporte des preuves que les hypothèses d'espèces fondées sur la morphologie de l'érable à sucre et de l'érable noir sont confirmées par la génétique, ces morpho-espèces correspondant à des groupes génétiques distincts. De plus, ces taxons semblent présenter un degré d'isolement reproducteur suffisant pour garder une cohésion génétique même dans un contexte de proximité géographique. Cela suggère que les concepts d'espèce biologique et de cohésion sont également respectés par l'érable à sucre et l'érable noir. Par ailleurs, les différences écologiques de ces deux taxons, qui ont été évaluées dans cette thèse par les différences de communautés herbacées qui leur sont

associées et qui sont documentées dans la littérature par d'autres mesures physiologiques (Hauer et al., 2021; Hilaire & Graves, 1999), supposent qu'ils répondent aussi au concept écologique d'espèce. Ainsi, les résultats de cette thèse montrent que ces taxons répondent à plusieurs critères de délimitation des espèces, ce qui justifie une réévaluation de leur nomenclature. En effet, la désignation de ces taxons comme sous-espèces dans certaines nomenclatures très citées (de Jong, 2002; van Gelderen et al., 1994) ne corrobore pas avec le respect de différents concepts d'espèces. Par exemple, au sein d'autres complexes d'espèces arborées la différenciation génétique entre des taxons désignés comme des sous-espèces n'est pas soutenue par la morphologie (*Ulmus minor;* Tamošaitis et al. 2021; *Eucalyptus globulus,* Jones et al. 2013).

4.1.2 Divergence

Selon la définition du continuum de spéciation proposée par Stankowski et Ravinet (2021), le processus de spéciation est intimement relié à la mise en place de barrières reproductives (*i.e*. à l'isolement reproducteur des populations). Ces barrières peuvent être prézygotiques (temporelles, géographiques, mécaniques, comportementales), ou postzygotiques (faible valeur sélective des hybrides). Bien que cette thèse apporte des preuves pour une distinction génétique entre les membres de la série *Saccharodendron*, les causes de leur divergence restent encore très peu connues. D'après la littérature, *A. skutchii* et *A. grandidentatum* auraient divergé par vicariance mais la divergence entre les autres taxons, dont les distributions se chevauchent largement, semblent avoir été influencée par l'effet d'une pression de sélection divergente (*i.e.* adaptation à différents environnements) (Areces-Berazain et al., 2021; Jackson et al., 2021; Vargas-Rodriguez & Platt, 2012). L'étude phylogénétique réalisée dans cette thèse n'associant aucune données morphologiques, phénologiques ou écologiques aux résultats phylogénétiques, elle ne permet donc pas de discuter des causes de la divergence de ces taxons. Une étude comparée des traits anatomiques, écologiques ou phénologiques pourrait aider à mettre en évidence les différentes barrières reproductives ayant participé à la divergence de ces taxons (Anacker & Strauss, 2014; Johannesson, 2001).

4.1.3 L'hypothèse du syngaméon

De nos jours, le continuum de spéciation n'apparaît plus comme un phénomène unidirectionnel dont l'apothéose sont des espèces non-inter-fertiles (Barberousse & Samadi, 2010; Cannon, 2021; Nosil, 2008). En effet, les analyses à l'échelle du génome révèlent aujourd'hui que l'hybridation interspécifique est relativement courante parmi les taxons, avec une prévalence estimée à 25 % chez les plantes et 10 % chez les animaux (Mallet, 2005). Lorsqu'un groupe d'espèces par ailleurs distinctes sont liées par hybridation, elles forment une communauté copulative appelée « syngaméon » (Buck & Flores-Rentería, 2022). Des syngaméons, aussi désignés sous le terme de « complexes d'espèces » dans la littérature, ont été observés chez différents genres animaux (e.g. *Canis*, *Desmogathus*) et végétaux (e.g. *Aesculus*, *Quercus*) (Cannon & Petit, 2019; dePamphilis & Wyatt, 1989; Pyron et al., 2020; Rutledge et al., 2010). L'origine des syngaméons est multifactorielle et il est possible que les taxons issus d'une radiation récente, comme c'est le cas pour ceux de la série *Saccharodendron*, soient plus enclin à former des syngaméons car les mécanismes d'isolement reproducteur sont faibles ou mal établis (Buck & Flores-Rentería, 2022). De ce fait, l'hypothèse du syngaméon semble valide à explorer pour la série *Saccharodendron*, en particulier pour les quatre espèces ayant apriori le plus de probabilité de s'hybrider naturellement du fait de leur proximité géographique : *A. floridanum*, *A. leucoderme*, *A. nigrum* et *A. saccharum*.

Parmi ces taxons, le couple formé par l'érable à sucre (*A. saccharum*) et l'érable noir (*A. nigrum*) constitue un cas d'étude central pour discuter l'hypothèse du syngaméon. Ces deux morphotypes coexistent fréquemment en sympatrie, présentent des traits morphologiques partiellement chevauchants et ont historiquement été décrits tantôt comme des espèces distinctes, tantôt comme des entités en cours de divergence ou des formes écologiques d'une même espèce. Dans cette thèse, les analyses génomiques réalisées sur des individus morphologiquement typiques de *A. saccharum* et *A. nigrum* ont mis en évidence l'existence de groupes génétiques distincts, y compris en contexte de sympatrie, suggérant le maintien d'une cohésion génétique propre à chacun de ces taxons malgré un fort potentiel de flux de gènes. Ces résultats indiquent que, si hybridation il y a, elle ne conduit pas à une homogénéisation complète des génomes, un schéma

compatible avec les attentes théoriques pour des espèces participant à un syngaméon tout en conservant leur identité génétique.

Cependant, à ce jour l'hypothèse selon laquelle les membres de la série *Saccharodendron* forment un syngaméon n'a pas été explorée et l'hybridation entre ces taxons est très peu connue. Elle est supposée fréquente basée sur l'observation de phénotypes morphologiques intermédiaires mais ses bases génétiques n'ont pas été explorées. Les travaux réalisés dans cette thèse se sont concentrés sur les formes « pures » des taxons de la série *Saccharodendron.* Les individus pouvant potentiellement représenter des hybrides au vu de leur morphologie intermédiaire entre deux formes « pures » ont systématiquement été mis de côté. Ce choix était motivé par le besoin de simplifier les échantillonnages et les questions de recherche : en se concentrant sur les morphologies typiques, le test des hypothèses d'espèces avec d'autres concepts d'espèces était simplifié. Bien que cette thèse ait identifié des groupes génétiques correspondant aux morphotypes de ces taxons, elle ne permet pas de documenter explicitement leur hybridation. La mise en œuvre d'études de génétique des populations sur ces espèces serait essentielle pour quantifier précisément les flux génétiques et évaluer les niveaux d'introgression entre taxons. L'introgression génétique, définie comme le transfert de matériel génétique d'une espèce à une autre par hybridation suivie de rétrocroisements répétés avec l'une des espèces parentales, peut conduire à l'incorporation stable d'allèles étrangers au sein de la diversité génétique d'une espèce, influençant ainsi son évolution et son adaptation (Anderson, 1953). Dans un syngaméon, il est attendu que des espèces s'hybridant de manière extensive présentent un certain degré d'introgression génétique (e.g. 5 à 20 % de composition génétique étrangère) tout en conservant une identité génétique distincte (*e.g.* chênes nord-américains; Hipp et al. 2019) . Ainsi, quantifier l'introgression au sein de la série *Saccharodendron* permettrait d'affiner notre compréhension des dynamiques d'hybridation et de statuer sur l'existence potentielle d'un syngaméon. Par ailleurs, les niveaux d'introgression génétique pourraient être corrélés à des données morphologiques afin d'éclairer l'origine des phénotypes intermédiaires observés chez plusieurs de ces taxons (*e.g.* saules; Fogelqvist et al. 2015). Enfin, des modélisations démographiques permettraient de reconstituer les événements passés à l'origine de la divergence de ces taxons et contribueraient ainsi à une meilleure compréhension de leur histoire évolutive (*e.g.* pins; Buck

et al. 2023). Une connaissance approfondie des mécanismes écologiques, phénologiques et géographiques sous-jacents à la divergence de ces taxons serait également déterminante pour mieux appréhender les facteurs assurant leur cohésion et leur maintien en tant qu'entités distinctes au sein d'un syngaméon où des échanges génétiques et des événements d'hybridation se produisent (Cannon & Petit, 2019).

4.1.4 Introgression adaptative

Cette thèse amène donc des éléments nouveaux quant à la distinction génétique des érables la série *Saccharodendron* tout en soulevant plusieurs questions quant au degré d'introgression génétique entre espèces. L'hybridation potentielle entre ces taxons soulève également la question de l'introgression adaptative au sein de cet ensemble d'espèces. L'introgression adaptative est un processus évolutif par lequel des gènes d'une espèce sont introduits dans le patrimoine génétique d'une autre espèce puis retenus dans la population réceptrice en raison de leur avantage adaptatif (Arnold & Kunte, 2017). Ce phénomène joue un rôle clé dans l'évolution en permettant aux populations d'acquérir rapidement des traits bénéfiques sans nécessiter l'évolution *de novo* de ces traits par mutation et sélection. L'introgression adaptative est un phénomène fréquent dans les syngaméons. Par exemple, chez les chênes européens (*Quercus robur*, *Q. petraea*), de la variation génétique introduite par introgression est associée à plusieurs variables environnementales, notamment la température annuelle moyenne et les précipitations, ce qui suggère son rôle dans l'adaptation à l'environnement (Leroy et al., 2020). Des mécanismes similaires sont observés dans le règne animal; par exemple chez les loups (*Canis lupus*) et coyotes (*C. latrans*), le transfert de gènes affectant la morphologie et le comportement facilitent l'adaptation à des niches écologiques variées (vonHoldt et al., 2011). Dans le cas de la série *Saccharodendron*, les taxons concernés sont distribués dans des environnements variés et la circulation de variation génétique adaptée à ces différents environnements peut conférer un avantage aux espèces qui le composent, particulièrement dans un contexte de changements climatiques. Ainsi, les études futures portant sur la caractérisation des niveaux d'introgression de

ces taxons pourrait également servir à l'identification de variation génétique adaptative et documenter plus avant leur potentiel adaptatif (*e.g.* armérie; Villa-Machío et al. 2022).

4.2 Le potentiel adaptatif de l'érable à sucre

4.2.1 Adaptation locale avec flux de gènes

Le troisième chapitre de cette thèse portait une attention particulière à des populations du taxon emblématique de la série *Saccharodendron* : l'érable à sucre. Tout d'abord, cette étude a mis en évidence une faible structure génétique au sein des populations d'érable à sucre échantillonnées au Québec. Les espèces forestières de cette région du monde présentent généralement une structure génétique reflétant les évènements démographiques passés influencés par les oscillations climatiques du Quaternaire (Roberts & Hamann, 2015). Dans les populations d'érable à sucre examinées dans cette thèse, la répartition de la variation génétique n'indique pas l'existence de lignées distinctes qui résulteraient d'un isolement passé dans des refuges climatiques au cours des oscillations quaternaires. Cependant il est probable que l'échantillonnage, représentant uniquement le nord de l'aire de répartition géographique de l'espèce (*i.e.* Québec), ait influencé le patron de distribution de la variation génétique observé. L'inclusion de populations provenant de la partie sud de l'aire de répartition de l'espèce (*i.e.* États-Unis) aurait probablement pour conséquence de changer les patrons de distributions de la variation génétique et permettrait peut-être de trouver un signal plus fort de lignées ancestrales issues des évènements démographiques passés. Malgré tout, cet échantillonnage met clairement en évidence la répartition homogène de la variation génétique à l'échelle du Québec ce qui suggère des flux de gènes importants connectant les populations, probablement du fait du mode de pollinisation anémochore de l'espèce qui avait été démontré par les travaux de Roussy (2014). De plus, le troisième chapitre de la thèse présentée ici met en évidence de la variation génétique associée à des variables environnementales malgré les flux de gènes important entre populations. Cela suggère donc que des allèles sont sélectionnés dans les populations par l'action de pressions de sélection plus fortes que les taux de migration bruts entre populations (Feder et al., 2012, 2014;

Yeaman & Guillaume, 2009). L'adaptation locale en présence de flux de gènes avait déjà été observée chez d'autres espèces (Savolainen et al., 2007; Tigano & Friesen, 2016). Cette thèse suggère qu'il y a adaptation locale chez l'érable à sucre et propose des SNPs potentiellement sous sélection. Par ailleurs, la variation génétique adaptative identifiée dans cette étude était statistiquement plus fortement associée avec différentes variables climatiques, écologiques et pédologiques. Le troisième chapitre apporte donc des preuves des changements héréditaires induits par l'adaptation à l'environnement chez l'érable à sucre. De ce fait, les locus identifiés constituent de potentielles cibles de sélection pour l'adaptation des générations futures, participant ainsi au potentiel adaptatif de l'espèce. En documentant le potentiel adaptatif de l'érable à sucre, les résultats de cette thèse participent plus généralement à la compréhension de sa capacité d'adaptation dans un contexte de changements climatiques et ouvrent plusieurs perspectives prometteuses. Premièrement, la validation expérimentale des locus identifiés pourrait être réalisée via des jardins communs et des analyses transcriptomiques, afin de confirmer leur rôle fonctionnel dans l'adaptation locale (Cavender-Bares & Ramírez-Valiente, 2017; Hess et al., 2016; Sun et al., 2020). L'analyse approfondie de la base moléculaire des traits adaptatifs pourrait élucider les mécanismes biologiques sous-jacents et identifier des cibles fonctionnelles pertinentes pour la compréhension des processus évolutifs (de Miguel et al., 2014; Remington & Purugganan, 2003). Par ailleurs, une extension de l'étude à d'autres populations ou gradients environnementaux permettrait d'évaluer la généralisation des associations observées. Enfin, le développement de ressources génomiques, incluant des catalogues de locus adaptatifs et des outils de génotypage ciblé (*e.g.* capture de séquences contenant des locus sous sélection environnementale), faciliterait les recherches futures et les applications pratiques en conservation et gestion des populations. Par exemple, en se basant sur les associations génétique-environnement identifiées, il devient possible de calculer le décalage génomique, ou « *genomic offset* », une mesure permettant d'estimer le degré de désadaptation probable des populations sous différents scénarios climatiques futurs (Rellstab et al., 2021). Ces analyses aident à repérer les populations les plus vulnérables, nécessitant des interventions prioritaires (Lotterhos, 2024). De plus, les données issues de cette étude peuvent guider des stratégies de flux de gènes assisté, en introduisant des allèles adaptatifs dans des populations locales par

croisements, pour accroître leur résilience face à des environnements changeants (Aitken & Bemmels, 2016; Hermisson & Pennings, 2005). Ces approches, intégrant les connaissances génomiques, permettent une gestion proactive et adaptative des populations naturelles, visant à préserver leur diversité génétique et leur potentiel adaptatif dans un contexte de perturbations environnementales croissantes.

4.2.2 Diversité génétique préexistante

Parmi les populations étudiées dans le troisième chapitre de cette thèse, certaines était situées sur des gradients d'altitude. Ces paires de populations montraient une répartition de la diversité génétique suggérant que des pressions de sélection divergentes s'appliquaient localement. Ce patron de divergence locale le long d'un gradient physique (élévation) venait appuyer le signal d'adaptation locale observé à l'échelle de la province. De plus, les positions génétiques impliquées dans la divergence le long du gradient étaient très variables. Cette variation dans les positions ciblées par la pression de sélection exercée par le gradient d'élévation met en évidence la multiplicité des stratégies disponibles au sein de l'espèce, reflétant le rôle crucial de la diversité génétique préexistante pour l'adaptation locale à des environnements contrastés. Cependant, la significativité des associations entre variables environnementales et variation génétique n'a pas pût être calculée le long des gradients d'élévation du fait d'un échantillonnage non adéquat. En effet, les variables environnementales utilisées dans l'approche statistique étaient inférées à partir des coordonnées des populations. Comme les gradients altitudinaux étaient représentés par deux populations, la variance entre les variables environnementales le long du gradient était trop faible pour effectuer une analyse statistique robuste. Afin de tester plus avant ce signal d'adaptation le long du gradient d'élévation il serait intéressant de reproduire ce dispositif d'échantillonnage en récoltant un nombre approprié de mesures de variables environnementales afin de pouvoir analyser statistiquement les associations génétique-environnement le long du gradient d'élévation. Étant donné que l'érable à sucre pourrait être amené à migrer vers des altitudes ou des latitudes plus élevées avec les changements climatiques, ce genre d'analyse venant agrandir nos connaissances sur la capacité d'adaptation de l'érable à sucre le long de

gradient environnementaux (e.g. élévation) serait utile pour une gestion efficace des populations naturelles.

4.3 Conclusions

En conclusion, cette thèse comprend des travaux venant : (i) soutenir la monophylie des taxons appartenant à la série taxonomique *Saccharodendron*, (ii) démontrer la distinction génétique de l'érable noir, une espèce vulnérable au Québec et de l'érable à sucre et (iii) identifier des positions génétiques associées à des variables climatiques spécifiques chez l'érable à sucre. La somme de ces résultats amène une meilleure compréhension de l'ensemble de taxons proches de l'érable à sucre et soulève plusieurs perspectives, notamment la possibilité de description d'un syngaméon d'érables en Amérique du Nord. De plus, certains des taxons compris dans cet ensemble sont considérés vulnérables dans certains états américains et provinces canadiennes, ce qui implique des répercussions possibles de cette thèse dans le milieu de la conservation. Enfin, cette thèse apporte des éléments de connaissances sur le potentiel adaptatif de l'érable à sucre et soulèvent différentes avenues de recherche afin de pousser plus avant ces résultats. En effet, il semble que les populations d'érables à sucre au Québec montrent des niveaux de diversité élevés et que cette diversité génétique préexistante a permis une adaptation à des conditions climatiques locales contrastées. Cette thèse montre ainsi que l'érable à sucre dispose d'un potentiel adaptatif élevé pouvant participer à sa résilience face aux changements climatiques d'origine anthropique. La perspective de l'existence d'un syngaméon d'érables en Amérique du Nord semble particulièrement intéressante à explorer compte tenu des implications que pourrait avoir l'introgression adaptative entre ces taxons aux écologies variées sur la diversité génétique préexistante disponible pour leurs potentiels adaptatifs. Bien entendu, cette conclusion générale sur le potentiel adaptatif de l'érable à sucre et des taxons proches se base sur les résultats de cette thèse et ne prend pas en compte d'autres facteurs pouvant grandement influencer la persistance des organismes; par exemple, l'impact des invasions biologiques et les interactions avec les microbiomes foliaires et racinaires. La prise en compte de ces phénomènes permettrait de dresser un tableau plus complet de la capacité d'adaptation de l'érable à sucre.

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